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Dietary copper intake influences skin lysyl oxidase in young men

Moshe J. Werman, Sam J. Bhathena, and Judith R. Turnlund

Department of Food Engineering and Biotechnology, Technion-Israel Institute of Technology, Haifa 32000, Israel; Metabolism and Nutrient Interactions Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, Beltsville, Maryland; and Western Human Nutrition Research Center, Agriculture Research Service, San Francisco, California

The effect of low dietary copper on copper status and the copper-containing enzyme lysyl oxidase was studied in young men. The study was divided into three dietary periods. During the first period, subjects were fed 0.66 mg/day Cu for 24 days (marginal copper). The level of copper was dropped to 0.38 mg/day for the next 42 days (low copper) and they were repleted with 2.49 mg/day Cu for next 24 days. Skin biopsies were taken at the beginning of the study and at the end of each dietary period and lysyl oxidase was measured enzymatically. There was a 24% drop in activity when the dietary copper level was reduced from 0.66 to 0.38 mg/day. When the subjects were repleted with copper, there was a significant increase in the activity of lysyl oxidase. The activity reached the level observed before the subjects were fed the restricted copper diet. These data show that, in humans, lysyl oxidase activity declines when dietary copper intake is inadequate and suggests that the cross-linking of collagen may be modulated by dietary copper. Lysyl oxidase in healthy young men can serve as a useful indicator of copper status. (J. Nutr. Biochem. 8:201–204, 1997) © *Elsevier Science Inc. 1997*

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Introduction

Copper is an essential trace element for various biological and physiological functions and structural integrity of various tissues in animals and humans.[1] The symptoms of copper deficiency in animals are well recognized. Although overt copper deficiency in humans is rare, there are two well-recognized genetic disorders of copper metabolism in humans; namely, Wilson's disease and Menke's disease. Symptoms of relative copper deficiency have been observed in humans.[2–5] It is estimated that the average intake of copper by the general population is lower than the estimated safe and adequate intake for adults which are 1.5 to 3.0 mg/day.[6]

The role of copper in collagen cross-linking is well established. The initiation and regulation of collagen cross-linking are through lysyl oxidase, which is a cuproenzyme.[7,8] Copper deficiency in animals results in low lysyl oxidase activity in heart, aorta, connective tissue, lung, and skin,[9–13] which increases with copper supplementation.[13–15] In chicks, copper deficiency has been shown to decrease cross-linking in bones, resulting in increased bone fragility.[16–18] Decreased lysyl oxidase activity seems to be responsible for bone defect in copper deficiency.[16–18] In Menke's disease, lysyl oxidase activity in the skin and aorta is markedly decreased secondary to the disturbances in copper metabolism.[19,20]

Copper has been shown to be essential for normal cardiac function.[21–23] Copper deficiency has been reported to cause changes in several metabolic risk factors associated with heart disease such as elevated fasting plasma triglycerides, cholesterol, and uric acid.[3,24] Some of the heart-related abnormalities observed in copper-deficient animals include cardiac edema, fibrosis, hypertrophy, rupture, arterial fibrosis, necrosis, degenerated elastin, focal myocardial hemorrhage, and ventricular aneurism. Some of these abnormalities in copper deficiency may be because of abnormal collagen and elastin. Cardiac collagen is extremely insoluble and highly cross-linked. Nutritional copper deficiency has been reported to cause marked changes in the metabolism of collagen in the myocardium and elastin of the cardiovascular system.[9,10]

Address reprint requests to Dr. Sam J Bhathena, Metabolism and Nutrient Interactions Laboratory, Beltsville Human Nutrition Research Center, Building 307, Room 324, Beltsville, MD 20705 USA.
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655 Avenue of the Americas, New York, NY 10010

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Table 1 Characteristics of subjects used for the study (11 males)

	Mean ± SD	Range
Age (yrs)	26 ± 4	21–32
Height (cm)	181 ± 6	173–189
Weight (kg)		
Start	74.3 ± 8.2	62–89
End	74.1 ± 7.9	62–87
Quetelet's index	22.7 ± 1.8	20.7–24.9
Energy intake (MJ)	12.9 ± 1.5	10.0–17.5

In this study, we tested the hypothesis that skin lysyl oxidase activity in healthy young men declines when dietary copper is inadequate and is restored with copper supplementation.

Methods and materials

The study protocol was approved by the Bionetics Institutional Review Board and by the U.S. Department of Agriculture Human Studies Review Committee. Twelve healthy males aged 21 to 32 years were recruited from the San Francisco area for the study. The selected participants were nonsmokers and had no history of cardiac problems or abnormalities, were not using prescription medication, and were willing to reside in the metabolic research unit for the duration of the study. Eleven men completed the study. The characteristics of the subjects selected are given in *Table 1*. All subjects were fed diets that were nutritionally complete in all nutrients except copper. All meals were prepared in the metabolic research facility of Western Human Nutrition Research Center. A 3-day menu cycle formulated from commonly available foods that are low in copper was used to ensure variety and acceptability.[25] Periodic caloric adjustments were made to maintain constant body weight. Copper content of the diet was adjusted by adding copper to diet. The study was 90 days in duration and was divided into three periods of 24 days, 42 days, and 24 days. During period one the subjects were given copper at 0.66 mg/day (marginal copper). During period two, the dietary copper was reduced to 0.38 mg/day (low copper). During the third period (repletion period), the subjects were given copper at the level of 2.49 mg/day (adequate copper).

Skin biopsies were taken at the start of the study (baseline) and at the end of each dietary copper period. The biopsy was taken from the buttocks with a 6-mm disposable biopsy needle containing approximately 50 mg of skin tissue. The wound was closed with sterile adhesive strips and covered with a Band Aid. The tissue was stored for 4 to 6 weeks at −70°C until analyzed. Copper status was assessed by measuring plasma copper, ceruloplasmin concentration, and ceruloplasmin activity at the same time as skin biopsies were taken. Urinary copper was measured throughout the study.

Lysyl oxidase from skin was extracted and measured as described previously.[26] Briefly, 50 mg of skin tissue was homogenized with 6 M urea and 0.16 M NaCl in 0.1 M phosphate buffered saline (PBS), pH 7.4, at 4°C to give a 5% homogenate. The homogenate was stirred for 2 hr at 4°C and then centrifuged at 135,000 × g for 45 min. The supernatant was dialyzed for 24 hr against several changes of PBS to remove the urea, and then centrifuged at 20,000 × g for 15 min at 4°C. The protein content of the supernatant was measured according to Lowry et al.[27] and was adjusted to 2 to 3 mg/mL. Soluble collagen substrate for measuring lysyl oxidase was prepared from tibial, femoral, and calvarial bones of 17-day-old chick embryos (kindly provided by Perdue Farms, Salisbury, MD USA) as described previously.[26]

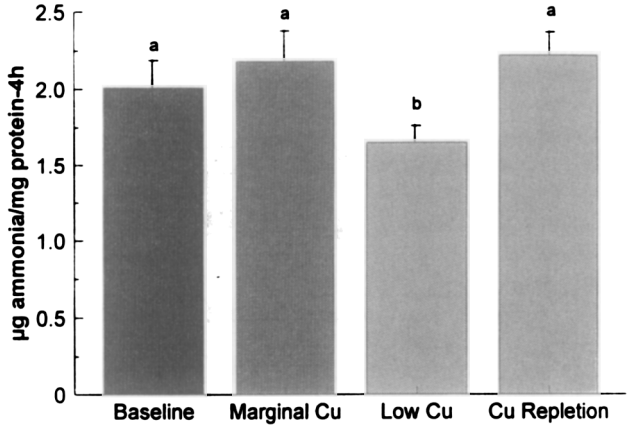

Figure 1 Lysyl oxidase activity in the skin of healthy male subjects fed different amounts of copper. Bars with different letters differ significantly ($P < 0.05$). Values are mean ± SEM of 11 subjects at each time period.

Lysyl oxidase activity in the skin extracts was measured colorimetrically as described previously.[26] The colorimetric method is more sensitive than the method that uses the release of radioactivity from labeled substrates.[26] The specificity of the assay to measure lysyl oxidase from rat skin and other tissues was ascertained by using β-aminopropionitrile (BAPN),[26] an irreversible inhibitor of lysyl oxidase in rats.[8,28]

The data were analyzed using PROC GLM in SAS/STAT.[29] The model for each variable was a randomized complete block design, where period was the treatment and subject was the blocking factor. The results were checked to ensure that the assumptions of the general model were met for each variable. The means for the periods were compared for each variable.

Results

The changes in skin lysyl oxidase activity with the changes in dietary copper intake are shown in *Figure 1*. There was no significant difference in lysyl oxidase activity between baseline value and after feeding 0.66 mg/day copper. However, there was a significant decrease in lysyl oxidase activity when subjects were fed 0.38 mg/day copper. There was a significant increase in the activity of the enzyme when the men were repleted with 2.49 mg/day of copper. The copper status of men receiving different amounts of copper in their diet was studied by measuring plasma copper and ceruloplasmin and urinary copper excretion.[30] All three indices were significantly lower ($P < 0.05$) at the end of the low dietary copper period compared with either the baseline or marginal copper period. On repletion with copper, all three parameters increased significantly compared with the levels observed at the end of the low copper period. Plasma copper fell from 13.8 µmol/L during marginal copper period to 12.6 µmol/L during depletion and increased to 13.6 µmol/L during repletion. Ceruloplasmin concentration fell from 284 to 258 mg/L, then increased to 280 mg/L. Urinary copper declined from 0.177 to 0.136 µmol/day then increased to 0.203 µmol/day. The decrease with depletion was significant, but the increase with repletion was not significantly different from either baseline or depletion.[30]

Discussion

In the present study, we observed significant decrease in the activity of lysyl oxidase in skin when subjects were fed a low copper diet, but not when they were fed diets marginal in copper, and the activity increased when subjects were replenished with adequate copper. Parameters of copper status, namely plasma copper, ceruloplasmin concentration and urinary copper declined when the dietary copper intake was lowest (0.38 mg/day). There were significant decreases in these parameters when the dietary level was reduced, followed by increases when the subjects were replenished with copper.[30] However, the increase in ceruloplasmin after copper replenishment was not significant.[30] Thus, lysyl oxidase in combination with other indices of copper status can be used to assess copper status in normal human subjects. Immune response measured by proliferation of mononuclear cells stimulated by mitogen was also decreased by the low copper diet in the same subjects.[25] Ceruloplasmin and superoxide dismutase are used as indices of copper status,[2,31–33] but they are not consistently reliable and do not always follow dietary copper intake.[34–36] In some disease states, plasma copper and ceruloplasmin levels are increased, that could mask copper deficiency.[5] Other copper containing enzymes, plasma diamine oxidase,[37] and peptidylglycine-α-amidating monooxygenase (PAM)[38] and plasma opioid peptides, especially enkephalins[39] may also be influenced by copper status. In diseased states, however, the activities of copper-dependent enzymes may not always reflect copper status. Recently, Percival et al.[40] reported increased plasma copper, but decreased SOD activity in neutrophils and lymphocytes of cystic fibrosis patients.

Lysyl oxidase is essential for the normal maturation of collagen, particularly in steps involving the formation of lysine-derived cross-links. In copper deficiency, skeletal abnormalities and cardiac abnormalities are present. A possible cause seems to be a decrease in the number of collagen cross-links,[8,17,18,41] which may be caused by decreased lysyl oxidase activity.[11]

It is not clear whether changes observed in skin lysyl oxidase also occur in heart lysyl oxidase. In several studies[10,11,13,42] changes in lysyl oxidase in several tissues, including the heart, reflect changes in plasma copper and/or the intake of dietary copper. However, tissue differences in the activity of lysyl oxidase have also been reported. Farquharson et al.[9] reported decreased collagen cross-linking in heart and femoral diaphysis, but not in the aorta and tibial diaphysis of copper-deficient male and female rats as compared with copper-adequate rats. A decrease in the lysyl oxidase activity in the aorta of copper-deficient rats[13] and pigs[12] and in plasma of copper-deficient pigs[12] has been reported. Similarly, forms of lysyl oxidase in human placenta, bovine lung, and bovine aorta may also be different from each other.[9]

It is important to note that although lysyl oxidase is involved in collagen cross-linking and therefore structural integrity of the collagen, a small reduction, though significant, may not be of biological importance. Thus, in chick bone normal cross-linking of collagen occurs with decreased lysyl oxidase activity[18] and more than 50% decrease in lysyl oxidase activity is required before a significant impairment in collagen cross-linking occurs.[43]

It is well established that copper plays an important role in cardiac function. In animal studies, producing copper deficiency by dietary means has been reported to cause significant decrease in lysyl oxidase activity in the heart.[9,10,12] There is a concomitant increase in soluble and total collagen and a decrease elastin in heart.[9,10,44] The effects of copper deficiency on myocardial collagen metabolism are more marked than those in other tissues.[9]

In summary, skin lysyl oxidase reflects changes in dietary copper level and copper status in young men and could be used as a marker for copper status in young men. This needs to be confirmed in women and older subjects. Further, because skin biopsy is an invasive procedure, the utility of lysyl oxidase as marker of copper status in population at large is limited. The cardiac abnormality of copper deficiency may be in part because of a defective collagen structure caused by decreased cross-linking as assessed by decreased lysyl oxidase activity, though a direct correlation between skin and cardiac lysyl oxidase activity needs to be clearly demonstrated in animal studies.

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