Biol Trace Elem Res (2013) 156:1–4
DOI 10.1007/s12011-013-9823-4

# Copper Supplementation at 8 mg Neither Affects Circulating Lipids nor Liver Function in Apparently Healthy Chilean Men

**Loreto Rojas-Sobarzo · Manuel Olivares · Alex Brito · Miriam Suazo · Magdalena Araya · Fernando Pizarro**

Received: 5 June 2013 / Accepted: 11 September 2013 / Published online: 6 October 2013


**Abstract** Copper (Cu) deficiency has been reported to influence lipid metabolism, but the effects in humans are controversial. To evaluate the effects of 8 mg Cu/day supplementation (as copper sulfate) for 6 months on the lipid profile and hepatic function of apparently healthy men. The design was randomized double-blind placebo-controlled clinical trial. Subjects and methods: 60 apparently healthy males aged 18–51 years were randomly assigned to Cu supplementation ($n=30$) or placebo ($n=30$). There was a nonsignificant reduction of 17 % in total cholesterol in both groups after supplementation. A 23 % nonsignificant reduction was observed in LDL cholesterol levels in the supplemented group. There was a nonsignificant increase of HDL cholesterol of 47 and 66 % in the control and supplemented groups, respectively. Triglyceride levels over 150 mg/dl were found in 17 subjects supplemented and 13 controls at baseline and decreased after supplementation to seven and eight subjects, respectively. There were no effects on serum Cu concentration or ceruloplasmin (protein) and hepatic transaminases. Supplementation of 8 mg Cu for 6 months had no effect on lipid profile of apparently healthy Chilean men with adequate Cu status.

**Keywords** Copper · Cholesterol · LDL · Lipid profile · Micronutrients

L. Rojas-Sobarzo · M. Olivares · A. Brito · M. Suazo · M. Araya · F. Pizarro (✉)
Institute of Nutrition and Food Technology (INTA), University of Chile, Av. El Líbano 5524, Macul, Santiago, Chile
e-mail: fpizarro@inta.cl

A. Brito
United States Department of Agriculture, Western Human Nutrition Research Center, University of California Davis, Davis, CA, USA

## Introduction

Copper (Cu) is an essential metal that participates as part of the catalytic centers of a series of enzymes with oxide–reductase activity. These include enzymes such as cytochrome-c-oxidase, superoxide dismutase, catechol oxidase, ceruloplasmin, and others. Thus, Cu is involved in fundamental processes such as cellular respiration, antioxidant defense, melanin pigment synthesis, connective tissue formation, and iron metabolism [1]. Cu deficiency and Cu excess are rather infrequent conditions in humans and have been reported mostly in individuals with genetic disorders (Menkes disease) [2] and Wilson disease [3]. Cu deficiency has also been identified in premature infants and in malnourished children [4]. Administration of Cu-deficient diets to human adults has been associated with low serum Cu concentration and an increase of total and LDL cholesterol during the depletion period [5–7]. The use of Wilson's disease as a model to study the effects of Cu excess in humans has also shed light on the relationship between Cu and lipid metabolism, showing that the accumulation of Cu in the liver has adverse effects on lipid metabolism. Cu excess alters gene expression, affecting the synthesis of cholesterol as well as the expression of enzymes involved in fatty acid metabolism and bile acid synthesis causing a ~30 % decrease of hepatic cholesterol and modifications in circulating lipids [8]. These data suggest high sensitivity of lipid metabolism to changes in Cu status and a direct link between both metabolisms. Consistent with this, several studies have shown that Cu deficiency increases plasma levels of cholesterol and triglycerides [9, 10], while in Cu-deficient mice supplementation with Cu decreases both concentrations [11]. The effect of milder forms of Cu deficiency or excess in humans is not clear. There have been few studies supplementing Cu to humans, and their results are controversial. Among other reasons, this may be due to the differences in the quality of individuals assessed (clinical patients vs community dwelling

subjects), the variable amounts of supplemented Cu, time of exposure, and methodological designs [12–15]. In this study, the objective was set at evaluating the effects of chronic daily supplementation with a Cu dose below but close to the upper level [16] on the lipid profile and liver function of apparently healthy men living at home and carrying out their customary daily activities.

## Subjects and Methods

*Design* This was a randomized double-blind placebo-controlled clinical trial; 60 apparently healthy males aged 18 to 51 years were randomly assigned to placebo ($n=30$) or Cu supplementation ($n=30$) for 6 months. Candidates that answered a poster calling for volunteers were invited to participate, and those who accepted signed an informed written consent and were enrolled in the protocol. Data regarding their clinical and dietary history was obtained, individuals were weighed, height was measured, and a baseline venous blood sample was obtained from the antecubital vein. The Cu supplemented group ingested daily, under supervision, a capsule containing 8 mg Cu (as Cu sulfate) and the control group a placebo of similar appearance, both for a period of 6 months. After the 6-month supplementation period, a second blood sample was obtained and determinations were repeated.

*Inclusion Criteria* Apparently, healthy men without firm diagnoses, who were asymptomatic during the last 6 months prior to this protocol, who did not consume vitamin/mineral supplements or any other medication on a chronic basis.

*Ethical Approval* The study was approved by the IRB of the Institute of Nutrition and Food Technology, University of Chile which follows the Helsinki Declaration.

*Biochemical and Protein Analyses* These were performed at the incorporation of the study and after the 6-month copper supplementation period. For the final sample, participants stopped consuming copper and placebo capsules 48 h prior to blood sampling. Serum copper (PerkinElmer, Model SIMAA 6100, The Perkin-Elmer Corporation, Norwalk, CT, USA), plasma ceruloplasmin (nephelometry, Array Protein System, Beckman Instruments Inc., Brea, CA), erythrocyte superoxide dismutase activity (Bioxytech SOD-525 Assay, Oxis Health products Inc, Portland, OR, USA), aspartate transaminase (AST/SGOT), alanine transaminase (ALT/SGPT), and gamma glutamyl transpeptidase (GGT) and lactate dehydrogenase (LDH) activities (Applied Clinical Chemistry SA, Amposta, Spain), triglycerides (DIALAB, triglycerides GPO-PAP kit), total cholesterol (DIALAB, cholesterol CHOD-PAP kit), HDL cholesterol (DIALAB, cholesterol-HDL precipitation kit), and LDL cholesterol (based on Friedewald formula) were measured.

### Definitions of "Normal" Values for Biochemical Indicators

The normal limit used for total cholesterol was defined at ≤200 mg/dl, for LDL cholesterol ≤130 mg/dl, and for triglycerides ≤150 mg/dl. For HDL cholesterol, the limit was set at ≤40 mg/dl determined according to the National Cholesterol Education Program adult treatment panel III [17]. Liver parameters were considered normal between the following ranges: 10–59 IU/L for AST, 13–40 IU/L for ALT, 2–30 IU/L for GGT, and 100–190 IU/L for LHD. For Cu serum and ceruloplasmin, the lower limit of the normal ranges were 70–140 μg/dL and 20–60 mg/dL, respectively [18].

*Dietary Intake Estimation* A 24-h recall and food frequency consumption questionnaires [19] were applied to estimate the dietary intake of copper [20]. We compared the reported intakes with the estimated average requirements, equivalent to 700 μg/day of Cu, by age and sex [21]. Dietary adequacy was defined as between 90 and 110 % of the reference intake.

*Statistical Analysis* Comparisons of groups were performed using Student's $t$ test for normally distributed variables and the nonparametric Mann–Whitney test for those variables without normal distribution. Effects of treatment were assessed by ANOVA for repeated measures (supplementation and time) for variables with normal distribution, and the nonparametric Kruskal–Wallis test was used for variables not normally distributed. The statistical significance level was set at $p<0.05$. All statistical analyses were performed with the statistical software STATA 12.0 (Stata Corp, College Station, TX, USA).

## Results

Participating individuals maintained their daily activities throughout the study period and showed no adverse effects associated with the protocol. There were no significant differences between general characteristics across groups (Table 1). Mean adherence to the study was 94 %; one participant was excluded from analysis because he did not follow and complete the protocol requirements. All subjects had normal serum Cu concentration (>70 μg/dl) at baseline and at the end of the study.

At baseline, blood concentrations of cholesterol were above 200 mg/dl in 33 and 35 % of supplemented and control individuals, respectively, without significant differences between groups. At the end of the study, these figures decreased by 17 % in both groups. With respect to LDL cholesterol levels, 37 % of the supplemented and 31 % of controls had levels higher than 130 mg/dl, which diminished nonsignificantly in the supplemented group by 23 %. Eighty-seven percent of the supplemented subjects and 90 % of controls had HDL cholesterol lower than 40 mg/dl at baseline which did not significantly

**Table 1** General characteristics of participants at baseline

| Characteristics | Total $n=59$ | Supplemented $n=30$ | Control $n=29$ |
|---|---|---|---|
| Age (years)[a] | 32±10 | 31±9 | 33±10 |
| Weight (kg)[a] | 73±12 | 73±12 | 73±12 |
| Height (cm)[a] | 169±6 | 170±0.1 | 171±0.1 |
| BMI (kg/m$^2$)[a] | 25±4 | 25±4 | 25±4 |
| Dietary intake copper estimation (mg/d)[b] | 1.39 (0.53) | 1.39 (0.50) | 1.37 (0.52) |
| Serum copper (μg/dl)[a] | 100±12 | 99±12 | 102±11 |

*BMI* body mass index

[a] Mean and standard deviation (X±SD), *t* test not significant for any variable

[b] Median and interquartile range, two-sample Wilcoxon rank-sum (Mann–Whitney) test not significant

increase after supplementation (47 and 66 %, respectively). Finally, triglyceride concentrations over 150 mg/dl were found in 17 supplemented subjects and 13 controls, which decreased after supplementation to seven and eight subjects, respectively. ANOVA analysis revealed no effect of time and supplementation on lipid profile after 6 months of supplementation (Table 2). There were no observed effects of supplementation on Cu status (Table 3) and liver transaminases, which were the indicators of liver function (Table 4).

## Discussion

Supplementing 8 mg of Cu for 6 months to apparently healthy Chilean men maintaining their customary daily activities did not produce significant changes on their circulating lipids, liver function, and blood Cu indicators. This is relevant considering that self-administered Cu supplementation (among other minerals and vitamins) is a common practice today in western society, and this has raised concern among health researchers about its potential adverse effects in the general population. At baseline, serum Cu concentrations were normal in all subjects and the average intake of dietary Cu conducted at baseline was 1.4 mg daily fulfilling daily recommendations of the metal [21]. Thus, we interpret the lack of significant changes as the group assessed had normal Cu status and the supplementation used was safely handled by the physiological homeostatic mechanisms. The fact that individuals receiving placebo showed trends of changes in the same direction as those receiving Cu supplements may reflect seasonal variations, a feature observed in several of our previous studies (unpublished).

As previously mentioned, studies on Cu supplementation and the lipid profile of humans are scarce and results are controversial. Alarcón-Corredor et al. [13] administering 5 mg of Cu for 45 days found a significant decrease in total blood cholesterol, LDL cholesterol, and plasma triglycerides, and there was a slight increase in HDL cholesterol. However, Alarcón-Corredor et al. conducted their study in hyperlipidemic subjects, making their results not comparable with ours.

**Table 2** Lipid profile before and after supplementation

| Parameters | Supplemented $n=30$ | | Placebo $n=29$ | |
|---|---|---|---|---|
| | Before *n* (%) | After *n* (%) | Before *n* (%) | After *n* (%) |
| Total cholesterol (mg/dl)[a] | 184±39 | 172±36 | 178±52 | 174±44 |
| HDL cholesterol (mg/dl)[b] | 31 (10) | 41 (13) | 31 (6) | 37(10) |
| LDL cholesterol (mg/dl)[a] | 119±34 | 110±39 | 107±41 | 112±37 |
| Triglycerides (mg/dl)[b] | 137 (109) | 113 (93) | 189 (189) | 106 (68) |

*HDL* high-density lipoprotein, *LDL* low-density lipoprotein

[a] Mean and standard deviation (X±SD), two factors ANOVA for repeated measures not significant

[b] Median and interquartile range, Kruskal–Wallis equality-of-populations not significant

**Table 3** Copper status biomarkers and antioxidant enzymes before and after supplementation

| Parameters | Supplemented $n=30$ | | Placebo $n=29$ | |
|---|---|---|---|---|
| | Before *n* (%) | After *n* (%) | Before *n* (%) | After *n* (%) |
| Serum copper (μg/dl)[a] | 102±11 | 102±11 | 99±12 | 101±12 |
| Serum ceruloplasmin (mg/l)[a] | 38±7 | 34±7 | 37±7 | 32±5 |
| E SOD (Umg/Hb)[b] | 95 (35) | 72 (22) | 93 (19) | 75 (15) |

*SOD* superoxide dismutase

[a] Mean and standard deviation (X±SD), two factors ANOVA for repeated measures not significant

[b] Median and interquartile range, Kruskal–Wallis equality-of-populations not significant

**Table 4** Liver function indicators before and after supplementation

| Biochemical profile | Supplemented $n=30$ | | Placebo $n=29$ | |
|---|---|---|---|---|
| | Before | After | Before | After |
| AST/SGOT (IU)[a] | 15 (6) | 22 (7) | 13 (3) | 21 (7) |
| ALT/SGPT (IU)[a] | 17 (13) | 23 (12) | 14 (6) | 20 (9) |
| GGT (IU)[a] | 14 (24) | 15 (14) | 14 (13) | 13 (10) |
| LDH (IU)[a] | 120 (47) | 148 (49) | 119 (33) | 139 (41) |

*IU* International unit, *AST/SGOT* aspartate transaminase, *ALT/SGPT* alanine transaminase, *GGT* glutamyl traspeptidase, *LDH* lactate dehydrogenase

[a] Median and interquartile range, Kruskal–Wallis equality-of-populations not significant

Also, since their protocol lacked a control group, the results described may be due to variables other than Cu supplementation like lifestyle changes or seasonal variations. Another study by Medeiros et al. [14] found no significant effect of Cu supplementation on lipid profile mainly because subjects in the control group decreased the levels of total and LDL cholesterol, which coincides with our study, suggesting seasonal variations rather than an effect of Cu supplementation. However, in Medeiros' protocol, the amount of Cu administered was 2–3 mg/day and the time of supplementation was 12 weeks, making results again not comparable to our study. On the other hand, Jones et al. [12] and DiSivestro et al. [15] supplemented hypercholesterolemic adult men with 2 mg Cu/day for 4 and 8 weeks, respectively. In both studies, there were no changes in the concentrations of total, LDL, or HDL cholesterol. In the DiSilvestro study, there was a significant increase in the enzymatic activity of erythrocyte superoxide dismutase and ceruloplasmin, as well as a decrease in the plasmatic levels of oxidized LDL cholesterol. However, this study did not include the assessment of Cu status in the participating individuals, so it is not possible to evaluate results versus potential changes in Cu status. The results presented here were obtained in subjects without pathological conditions and without Cu deficiency, providing information about the effects that nutritional intervention and mineral supplementation may have rather than the usefulness of Cu in pathological conditions. In this context, it is important to highlight the study design which minimized systematic biases; the dose of Cu at 80 % of the upper level, having no adverse effects on the indicators evaluated and on the overall health of participants, and the duration of the study period, which to our knowledge is the longest Cu supplementation reported in healthy humans using Cu doses as described.

## Conclusions

Eight milligrams Cu supplemented for 6 months has no effect on the lipid profile of apparently healthy Chilean men with adequate Cu status, and also, it does not have adverse effects on general health, liver function, and Cu status.

**Acknowledgments** We would like to thank Angélica Letelier for his excellent technical assistance and Sotiris Chaniotakis for his work editing the English of this manuscript. This study was supported by the International Copper Association and FONDECYT no.1070665 and FONDECYT no.1110099.

## References

1. Uauy R, Olivares M, Gonzalez M b (1998) Essentiality of copper in humans. Am J Clin Nutr 67(5 Suppl):952S–959S
2. Tümer Z, Møller LB (2010) Menkes disease. Eur J Hum Genet 18: 511–518
3. Tao TY, Gitlin JD (2003) Hepatic copper metabolism: insights from genetic disease. Hepatology 37(6):1241–1247
4. Weisstaub G, Medina M, Pizarro F, Araya M (2008) Copper, iron and zinc status in moderately and severely malnourished children recovered following WHO protocols. Biol Trace Elem Res 124(1):1–11
5. Klevay LM, Inman L, Johnson LK et al (1984) Increased cholesterol in plasma in a young man during experimental copper depletion. Metabolism 33:1112–1118
6. Reiser S, Powell A, Yang CY, Canary JJ (1987) Effect of copper intake on blood cholesterol and its lipoprotein distribution in men. Nutr Rep Int 36:641–649
7. Turnlund JR, Scott KC, Peiffer GL, Jang AM, Keyes WR, Keen CL, Sakanashi TM (1997) Copper status of young men consuming a low-copper diet. Am J Clin Nutr 65(1):72–78
8. Huster D, Lutsenko S (2007) Wilson disease: Norjust a copper disorder. Analysis of a Wilson disease model demonstrates the link between copper and lipid metabolism. Mol Biosyst 3:816–824
9. Al-Othman AA, Rosenstein F, Lei KY (1993) Copper deficiency increases in vivo hepatic synthesis of fatty acids, triacylglycerols, and phospholipids in rats. Proc Soc Exp Biol Med 204:97–103
10. Al-Othman AA, Rosenstein F, Lei KY (1994) Pool size and concentration of plasma cholesterol are increased and tissue copper levels are reduced during early stages of copper deficiency in rats. J Nutr 124:628–635
11. Alarcón-Corredor OM, Carnevalí de Tatá E, Reinosa-Füller J, Contreras Y, Ramírez de Fernández M, Yánez-Domínguez C (2000) Changes in serum lipids in rats treated with oral cooper. Arch Latinoam Nutr 50:249–256
12. Jones AA, DiSilvestro RA, Coleman M, Wagner TL (1997) Copper supplementation of adult men: Effects on blood copper enzyme activities & indicators of cardiovascular disease risk. Metabolism 46:1380–1383
13. Alarcón-Corredor OM, Guerrero Y, Ramírez de Fernández M, D'Jesús I, Burguera M, Burguera JL, Di Bernardo ML, García MY, y Alarcón AO (2004) Effect of copper supplementation on lipid profile of Venezuelan hyperlipemic patients. Arch Latinoam Nutr 54:413–418
14. Medeiros D, Milton A, Brunett E, Stacy L (1991) Copper supplementation effects on indicators of copper status and serum cholesterol in adult males. Biol Trace Elem Res 30:19–35
15. DiSilvestro RA, Joseph EL, Zhang W, Raimo AE, Kim YM (2012) A randomized trial of copper supplementation effects on blood copper enzyme activities and parameters related to cardiovascular health. Metabolism 61:1242–1246
16. Barr SI, Murphy SP, Agurs-Collins TD, Poos MI (2003) Planning diets for individuals using the dietary reference intakes. Nutr Rev 61(10):352–360
17. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (2002) Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 106: 3143–3421
18. Duh S, Denis J (2005) Laboratory reference ranges values. Pp 98–112. http://www.stedmansonline.com/webFiles/Dict-Stedmans28/APP17.pdf. Accessed 14 May 2013
19. Rebolledo A (1998) Encuestas Alimentarias. Rev Chil Nutr 25: 28–34
20. Gattás V (2008) guía de composición nutricional de alimentos naturales, de la industria y preparaciones chilenas habituales. Universidad de Chile-INTA, Santiago
21. Copper (2006) Dietary reference intakes: the essential guide to nutrient requirements. The National Academies, Washington, DC