Differential response of interleukin-2 production to chronic copper supplementation in healthy humans

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ABSTRACT. Background. Copper (Cu) is an essential trace element for many biological processes including maintenance of both innate and acquired branches of immunity. Objective. To measure the effect of copper supplementation on IL-2 and TNF-α production in subjects with lower and higher ceruloplasmin (Cp) values within normal range. Design. Healthy adults (17 men and 16 women) with normal-low (low Cp) and normal-high Cp (high Cp) values were supplemented with 10 mg Cu/day (as CuSO4) during 2 months. Method. Before and after supplementation blood mononuclear cells were incubated in the absence or presence of phytohaemagglutinin or lipopolysaccharide for induction of IL-2 and TNF-α, respectively. The secretion of cytokines was measured by ELISA. Cu supplementation did not modify classical biochemical markers of Cu status. Results. After supplementation, a significant increase in IL-2 production was found only in subjects with normal-low plasma Cp. Before and after Cu supplementation geometric mean and range ± 1 SEM values were 1,566 (1,287-1,905) and 2,514 (2,159-2,927) pg/mL, respectively (two way ANOVA for repeated measures: Cp level p < 0.001; time = NS; interaction Cp level and time p < 0.05). We did not observe changes in TNF-α production after Cu supplementation. Conclusions. Cu supplementation increased secretion of IL-2 and not TNF-α, which suggests an activation of proliferative but not inflammatory cytokines. These results support hypothesis that IL-2 may be a good indicator to identify a subgroup of individuals (polymorphism) who differs in Cu metabolism.

Keywords: copper supplementation, serum copper, ceruloplasmin, IL-2, TNF-α, humans
the effects of a controlled 2-month copper supplementation on the production of IL-2 and TNF-α in individuals chosen from the = 5% of the upper and lower values of the Cp distribution curve obtained in 800 apparently healthy adults.

PATIENTS AND METHODS

Design and study group

This study is part of a larger protocol that assessed the response of several biochemical and urinary potential indicators of copper status to 10 mg of copper (as copper sulfate) administered as a single daily dose, for 60 days. Those results will be reported elsewhere. The 800 apparently healthy individuals screened for their serum ceruloplasmin protein were free of acute infectious/inflammatory processes (C-reactive protein in blood samples < 0.8 mg/dL), of chronic illnesses, of anemia (hemoglobin < 120 g/L for women and < 130 g/L for men,) and of chronic multi medication that may interfere with the study. Potential participants received detailed information about the protocol and those who accepted to participate signed an informed consent prior to starting copper supplementation. This consisted of two 5 mg copper gelatin capsules (as copper sulfate), administered in a controlled fashion once per day, with plain tap water, between meals. Blood samples obtained by venous puncture were obtained within 3 days prior to and after copper supplementation. Fourteen and 19 individuals of both gender, belonging to the lower (low Cp) and higher (high Cp) decile in the Cp distribution curve obtained, respectively, formed the study groups. Participants were 25 to 45 years of age, balanced by sex. The protocol and the consent form were approved with the study. Potential participants received detailed information about the protocol and those who accepted to participate signed an informed consent prior to starting copper supplementation. This consisted of two 5 mg copper gelatin capsules (as copper sulfate), administered in a controlled fashion once per day, with plain tap water, between meals. Blood samples obtained by venous puncture were obtained within 3 days prior to and after copper supplementation.

Copper status

This was assessed by means of plasma copper, plasma Cp and erythrocyte Zn-Cu-superoxide dismutase (eSOD). Plasma Cu was analyzed by atomic absorption spectrophotometry (Perkin Elmer Model 2280, Norwalk, Conn.), plasma ceruloplasmin protein was determined by nephelometry (Array Protein System, Beckman Instruments Inc., Brea, CA) and eZn-Cu SOD activity was measured using a commercial kit (Biosytech SOD-525 Assay, OXIS International Inc, Portland OR).

Cell studies

Blood mononuclear cells (BMNC) were isolated on Histopaque gradient (density: 1.119, Sigma Diagnostic, St. Louis, MO), washed twice with phosphate buffer saline (PBS) and suspended at 4.0 x 10^6 cells/mL in RPMI 1640 culture medium with gentamycin (Elkins-Sinn, Inc. Cherry Hill, NJ).

Induction of IL-2 and TNF-α

BMNC suspension was dispensed in 96-well flat-bottom microtiter plates (1 x 10^5/well) in the absence or presence of phytohaemagglutinin (PHA: 10 μg/mL) or lipopolysaccharide (LPS: 1.0 μg/mL) for induction of IL-2 and TNF-α, respectively. Cultures in triplicate were incubated for 1 day at 37°C in humidified atmosphere (95% air-95% CO2). Supernatants were collected and kept at -70°C until cytokine assays. Determinations were performed in one batch to avoid inter-assay variations [18]. In vitro production of IL-2 and TNF-α were measured by using specific commercial enzyme-linked immunosorbent assay (ELISA: R&D System, Minneapolis, MN). Results were expressed as mean picograms per milliliter (pg/mL) obtained in duplicate experiments.

Statistical analysis

Statistical analyses were performed using Statistica for Windows, release 4.5 (StatSoft Inc., Tulsa, OK) and included two way ANOVA for repeated measures and Pearson correlation. Because the values of IL-2 and TNF-α had a skewed distribution, these values were converted to logarithms before performing mean, SEM and statistical analysis. Results were retransformed into anti-logarithms to recover the original units and expressed as geometric means and range ± 1 SEM range.

RESULTS

Participants remained healthy along the study period; daily home visits controlled copper ingestion and appearance of symptoms, revealing that participants remained free of clinically apparent infectious and inflammatory processes. Table 1 shows the indicators used to define Cu status. As expected, prior to supplementation both serum Cu and serum Cp were significantly different between the study groups (p < 0.001) but eSOD activities were similar. In agreement with C reactive protein, total leukocytes count was normal in all groups at both times of assessment. After

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before supplementation</th>
<th>After supplementation</th>
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<tbody>
<tr>
<td></td>
<td>Low Cp (n = 14)</td>
<td>High Cp (n = 19)</td>
</tr>
<tr>
<td>Serum copper (μmol/L)</td>
<td>12.2 ± 4.2</td>
<td>24.5 ± 5.5</td>
</tr>
<tr>
<td>Ceruloplasmin (mg/L)</td>
<td>255 ± 22</td>
<td>603 ± 119</td>
</tr>
<tr>
<td>SOD Activity (U/mg Hb)</td>
<td>84.1 ± 18.9</td>
<td>86.7 ± 45.9</td>
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<td>Leukocyte (10^9/L)</td>
<td>7.2 ± 2.0</td>
<td>6.7 ± 1.3</td>
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</table>

Values expressed as mean ± 1 SD. SOD = superoxide dismutase. *p < 0.001 between groups before supplementation and after supplementation.
supplementation there were no significant changes of serum Cu, Cp protein or eSOD. IL-2 production by PHA-stimulated BMNC was similar in both study groups prior to copper supplementation (figure 1). After supplementation, a significant increase in IL-2 production was found only in the low Cp group (geometric mean and range ± 1SEM in pg/mL = 1,566 (1,287-1,905) to 2,514 (2,159-2,927) before and after Cu supplementation respectively (two way ANOVA for repeated measures: Cp value p < 0.001; time = NS; interaction Cp value and time p < 0.05). No statistical sex-differences on IL-2 production in low Cp subjects were found before and after supplementation. There are not secondary effects on IL-2 production due to zinc and iron status. Number of responders for increased cell output of IL-2 to the Cu supplement were 11/14 and 4/19 in low and high Cp groups, respectively. In addition, subjects with low plasma Cp showed a higher correlation (r = 0.82, p < 0.001) between IL-2 levels before and after nutritional intervention than those individuals with high Cp (figure 2). TNF-α production by LPS-stimulated BMNC in supernatants of cells from both groups were similar between the study groups both prior to and after copper supplementation. Before and after supplementation, mean (range) values were 3825 pg/mL (2516-6689) and 4135 pg/mL (2561-6109) in low Cp volunteers, and 4358 pg/mL (1571-8176) and 4235 pg/mL (1564-9487) in high Cp group.

### DISCUSSION

Results show that the 2-month Cu supplementation with 10 mg/day induced an increased of IL-2 but not of TNF-α production in individuals that represented the lower end of the Cp distribution curve obtained in apparently normal population. It calls the attention that this subgroup did not increase plasma levels of Cp after Cu supplementation. We only measured the protein concentration without evaluate the enzymatic activity. It is known that Cu deficiency affects preferentially Cp activity and in lower degree the protein concentration [19]. However, marginal copper deficiency in our volunteers was discarded since no changes in eSOD after Cu supplementation were found. A possible explanation is that individuals with low Cp represent a particular genetic polymorphism for driving Cu metabolism and they would have an increased oxidative stress, after copper supplementation that determines an increased IL-2 production. Recent studies demonstrated that oxidative products like hydrogen peroxide promoted IL-2 production in cultured Jurkat T cells [20]. Future investigation could clarify these alternatives.

It has been difficult to estimate dietary recommendations of copper. Former figures of 1.5-3.0 mg Cu/day [21] have been challenged because a large survey of copper content in the diet showed that up to 30% of the diets measured were below recommendations, however, no apparent copper deficiency was observed in the population [22]. Rec-
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REFERENCES


