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The aim of this study was to determine whether total copper or soluble copper concentration is associated with gastrointestinal signs and symptoms. Forty-five healthy adult women (18–55 years of age), living in Santiago, Chile, ingested tap water with 5 mg/L of copper containing different ratios of soluble copper (copper sulfate) and insoluble copper (copper oxide) over a 9-week period. Three randomized sequences of the different copper ratios (0:5, 1:4, 2:3, 3:2, and 5:0 mg/L) were followed. Subjects recorded their water consumption and gastrointestinal symptoms daily on a formal. Mean water consumption was similar among groups. Serum copper levels, ceruloplasmin, and activities of liver enzymes were within normal limits. No differences were detected between the means of biochemical parameters at the beginning and at the end of the study. Twenty subjects presented gastrointestinal disturbances at least once during the study, 9 suffered diarrhea (with or without abdominal pain and vomiting), and the other 11 subjects reported abdominal pain, nausea, or vomiting. No differences were found in incidence of abdominal pain, nausea, vomiting, and diarrhea regardless of the ratio of copper sulfate to copper oxide. In conclusion, both copper sulfate (a soluble compound) and copper oxide (an insoluble compound) have comparable effects on the induction of gastrointestinal manifestations, implying that similar levels of copper were present in the stomach. Key words: drinking water, gastrointestinal symptoms, insoluble copper, organic copper.

Materials and Methods

This study was randomized to copper exposure, blinded to the volunteers and supervisor, and was prospective in nature. The participants included 45 healthy, adult women (18–55 years of age), who worked at home throughout the day and who were neither pregnant nor lactating. They lived in urban Santiago, Chile, in a neighborhood constructed 16 years ago. Houses shared similar characteristics, and all had copper piping systems. Volunteers were reimbursed for the expenses they incurred to attend our facilities and for the monies they did not earn on the days of study. Volunteers were randomized into three groups (15 subjects each) based on the sequence of soluble/insoluble copper salt that they received following a Latin square design (Figure 1). The different proportions of copper sulfate (soluble) to copper oxide (insoluble) were 0.5, 1:4, 2:3, 3:2, and 5:0 mg/L. The total study duration was 9 weeks, which was divided into five 1-week study periods, with a 1-week break in between. The blinded nature of the study assured that subjects did not know exactly what soluble copper concentration they were receiving. The Latin square experimental design was used to control for potential time effects (e.g., becoming accustomed to the water) and confounding variables such as menstrual period, alcohol consumption, and food intolerance.

All participants received oral information about the objective and procedures of the study during an introductory meeting to which they were invited by means of local advertisements. Those who decided to participate signed an informed consent form before they were included in the study. The protocol was approved by the Ethics in Human Research Committee of the Institute of Nutrition and Food Technology of the University of Chile.

For every week of each study period the subjects received seven dark-polypropylene flasks filled with a solution to add to their home drinking water. Every morning each subject mixed the test solution contained in the flasks provided with tap water in a graduated polypropylene container 2 L in size. Subjects were instructed to vigorously shake the container before drinking the water or consuming infusions and beverages right after they are prepared. At the end of each day any remaining water in the container was discarded. At this time, every subject also registered on an ad-hoc form the amount of water ingested, the amount left in the container, and any symptoms experienced during the day (nausea, vomiting, abdominal pain or cramps, diarrhea, headache, joint pain, backache, change of appetite, etc.). During the nonstudy periods (break weeks) the subjects were also asked to record daily any gastrointestinal symptoms on a form.

Twice a week a field worker monitored the in-home preparation of the test water, evaluated the morbidity reported since the last site visit, and supervised the completion of questionnaires. Subjects who developed gastrointestinal symptoms were offered a 50% discount on their water bill.

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diarrhea, abdominal pain, or vomiting were instructed to stop consumption of the test water for the next 2 days. If the symptoms disappeared, the individual resumed consumption of the test water. If the symptoms reappeared, the subject was instructed to stop drinking the test water until the next study period began.

Once a week, the actual copper concentration and pH (HANNA Instruments, Model Checker 1, Woonsocket, RI, USA) of water prepared by the subjects in their homes were determined by examination of samples obtained by field workers during unexpected home visits. Soluble copper was measured directly by absorption atomic spectrophotometry in a sample of centrifuged water (Perkin Elmer M odel 2280, Norwalk, CT, USA; sensitivity = 0.01 µg/mL and variability = < 3%). Total copper was measured in the samples, previously mixed with HCl 1 N in a proportion of 1:1. The insoluble copper was defined as the total copper minus soluble copper. The concentrations of ionic copper (Cu²⁺) in the different CuO/CuSO₄ mixtures were determined under simulated gastric conditions (37.5°C and pH 2) using a cupric electrode (Orion, model 9629 ion plus series, Orion Research, Inc., Beverly, MA, USA).

The average maximum ion concentrations in drinking water of the city of Santiago are as follows: sulfate (280 mg/L), calcium (169 mg/L), sodium (46 mg/L), chloride (68 mg/L), magnesium (12 mg/L), fluoride (0.6 mg/L), and copper (0.33 mg/L) (13).

To evaluate changes in copper nutritional status associated with the copper administered during the study, we obtained blood samples 1 week before the beginning of the study and again at the end of the protocol. Serum copper was measured by atomic absorption spectrophotometry and ceruloplasmin by radial immunodiffusion (The Binding Site, Inc., The Netherlands; sensitivity = 0.1 µg/mL and variability = < 3%); total copper was measured from the samples, previously mixed with HCl 1 N in a proportion of 1:1. The insoluble copper was defined as the total copper minus soluble copper. The concentrations of ionic copper (Cu²⁺) in the different CuO/CuSO₄ mixtures were determined under simulated gastric conditions (37.5°C and pH 2) using a cupric electrode (Orion, model 9629 ion plus series, Orion Research, Inc., Beverly, MA, USA).

There were no significant differences in age, weight, and height of the subjects among the groups. The means and SDs for all the subjects were 25.6 ± 5.1 years, 62.7 ± 9.9 kg, and 1.60 ± 0.06 m, respectively.

Serum copper level activities of liver enzymes (ASAT, ALAT, and GGT) and ceruloplasmin were within the normal limits, but seven women were anemic (Hb < 120 g/L). No differences were detected between the means of biochemical parameters at the beginning and at the end of the study (Table 1).

The copper content of tap water was < 0.1 mg/L and therefore was not considered to be a significant source of copper. Mean water consumption was similar among groups (Figure 2). Seventy percent of subjects consumed > 1.0 L daily, despite the level of copper in the water, and only 3% ingested < 0.5 L water/day. Fluid intake showed little individual differences throughout the study (up to ± 0.3 L/day). Although not recorded in detail in this protocol, in all our previous studies among this type of population, one-third of total daily water intake is as plain water, one-third as home-prepared flavored drinks, and one-third as tea or coffee. Figure 3 shows the mean of soluble and nonsoluble copper measured in the samples of water prepared by the subjects. When the water was prepared with 0.5:1, 2:3, 3:2, and 5:0 ratios of copper sulfate:copper oxide (mg:mg per L), the means and SD of soluble copper measured from the samples were 0.1 ± 0.2, 0.9 ± 0.5, 1.8 ± 0.6, 2.7 ± 0.6, and 4.7 ± 0.8 mg/L, respectively. For total copper the means and SDs were 4.7 ± 1.4, 5.3 ± 1.6, 4.7 ± 1.9, 5.2 ± 0.6, and 5.1 ± 0.6 mg/L, respectively (F = 2.03; not significant).

When copper was measured at simulated gastric conditions, a high proportion of copper was ionic (Cu²⁺), regardless of the proportion of salts present in the drinking water. Percentages of Cu²⁺ ranged from 90% to 100% for all of the copper solutions studied.

### Results

Table 2 summarizes the symptomatology observed by concentration of soluble copper in drinking water. Twenty seven subjects presented gastrointestinal disturbances at least once during the study. Nine suffered diarrhea (with or without abdominal pain and vomiting), and the other 11 subjects reported abdominal pain, nausea, or vomiting. Expressing results by the ratio copper sulfate:copper oxide ingested oxide (0.5, 1.4, 2.3, 3.2, and 5.0 mg:milligrams per liter) 5, 3, 3, 2, and 6 episodes of gastrointestinal symptoms (excluding diarrhea) were reported, respectively (χ² = 3.03, not significant). No differences were found in incidence of diarrhea depending on the ratio of copper sulfate to copper oxide (χ² = 3.89; not significant).

Seven of the 10 episodes of diarrhea detected occurred in the first half of the study period. This was not the case with nausea and abdominal pain, which were homogenously distributed throughout the study period. Contrasting the symptoms reported when the subjects ingested 5 mg/L of added copper (in different proportion of copper sulfate:copper oxide) with those reported by the same subjects during the period when they ingested plain tap water (break weeks), the incidence of total gastrointestinal symptoms with 5 mg Cu/L was significantly higher (p < 0.01; Table 3).

### Discussion

Copper ingested from food and water is rarely harmful to humans because the content of ionic copper is relatively low. Food is the principal source of copper for humans unless copper-contaminated water is consumed. The copper content of potable water is low; however, acidic and hard water, especially if it is conducted by newly installed copper pipes, may be highly corrosive, thus increasing the copper concentration of water. Stagnation is another factor that will increase copper content of water, and should be considered when establishing sampling procedures. Copper can produce gastrointestinal symptoms by irritating the gut mucosa and/or altering the microbial flora of the colon. Animal studies have shown that vomiting induced by copper is mediated by serotonine gastric receptors. In addition, copper has a direct effect on gastric mucosa

### Table 1. Copper nutrition status, liver enzymes, and hemoglobin concentrations at the beginning and at the end of the study.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Final</th>
<th>Change (%)</th>
<th>p-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum copper (µg/dL)</td>
<td>129 ± 29</td>
<td>125 ± 32</td>
<td>-2.6</td>
<td>0.125</td>
</tr>
<tr>
<td>Ceruloplasmin (mg/dL)</td>
<td>40.4 ± 10.7</td>
<td>40.2 ± 10.8</td>
<td>-0.5</td>
<td>0.887</td>
</tr>
<tr>
<td>ASAT, U/L (range)</td>
<td>6.7 (13.3–5.6)</td>
<td>3.7 (11.0–2.6)</td>
<td>-50.2</td>
<td>0.060</td>
</tr>
<tr>
<td>ALAT, U/L (range)</td>
<td>7.3 (22.8–3.9)</td>
<td>4.9 (11.0–2.2)</td>
<td>-50.2</td>
<td>0.089</td>
</tr>
<tr>
<td>GGT, U/L (range)</td>
<td>6.1 (14.2–2.6)</td>
<td>4.9 (11.0–2.2)</td>
<td>-50.2</td>
<td>0.146</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>143 ± 15</td>
<td>144 ± 11</td>
<td>1.1</td>
<td>0.249</td>
</tr>
</tbody>
</table>

*All p-values were nonsignificant (t-test). **Geometric mean and range ± standard deviation.
nerve endings of the parasympathetic nervous system (14–16). Direct stimulation of the vomiting center in the central nervous system by absorbed copper ions may occur with acute ingestion of highly soluble copper salts (17), suggesting that the emetic effect is a protective response. We, as well as others, have demonstrated that copper in drinking water may cause gastrointestinal disturbances if the concentration is sufficiently high (18–21). However, most published studies have serious methodological problems, such as uncertainty of the copper concentration in the beverage or drinking water at the time of the episode of gastrointestinal symptoms, presence of confounding variables such as alcohol or nitrate intake associated with the episodes, and lack of information on the microbiological quality of drinking water or beverages that were involved in the episodes (10). In our recent study using a randomized, controlled design, we demonstrated a significant increase in nausea, abdominal pain, and vomiting in adult women receiving water with ≥ 3 mg Cu/L (11). In this previous study volunteers ingested copper salts only as plain water. Study subjects received a highly ionizable copper compound (CuSO₄) with high water solubility (316 g/L).

The present study was designed to address the following question: Can insoluble copper oxide induce gastrointestinal symptoms at the equivalent copper content of a copper sulfate solution? As representative of an insoluble compound, we chose cupric oxide (CuO) because it is the most common copper form added to vitamin and mineral supplements. The total concentration of copper was fixed at 5 mg/L, because at lower copper concentrations previous studies suggested that gastrointestinal complaints might be infrequent, making it potentially difficult to demonstrate statistically significant differences of the end points defined. Presumably the intragastric concentration of copper oxide varied depending on the sum of CuSO₄ and the proportion of copper oxide that became ionized in the acid gastric environment.

Comparing the gastrointestinal symptoms reported while ingesting plain or copper-added water, ingestion of water with 5 mg/L of added copper, resulted in a 4-fold increase in reported gastrointestinal symptoms (nausea, abdominal pain, vomiting, and diarrhea). The incidence of gastrointestinal symptoms, however, was not significantly different among the individuals receiving the different ratios of soluble/insoluble copper salts, thus disproving the hypothesis that mainly soluble copper compounds would be responsible for acute gastrointestinal symptoms. On the contrary, the similar prevalence of symptoms noted within the groups suggests that copper oxide is rapidly solubilized by the low intragastric pH. This proposition was supported by our in vitro studies, which demonstrated that at pH 2 > 90% of copper provided as copper oxide was ionized within 5 min.

Classic parameters of copper status and liver function did not change significantly. As in previous studies using a control exposure to 5 mg Cu/L, none of the subjects presented liver function tests beyond the normal range.

Because of the borderline increase (p = 0.06) observed on ASAT levels, case-by-case analysis was done; this showed that in none of the cases were the changes detected on enzyme activity clinically significant.

Six out of the 12 total episodes of diarrhea observed in the study occurred during the initial 7 days of this 2 month study, suggesting that copper tolerance develops over time. This is in agreement with our previous finding (when copper sulfate was tested at 1, 3, 5 mg/L as sulfate) of a time effect in diarrhea prevalence, independent of dose. The prevalence of diarrhea decreased over the initial 2 weeks (11). No time effect was observed for nausea, abdominal pain, and vomiting.

If we extrapolate our findings to the usual form of copper exposure (i.e., copper-contaminated water consumed with food), we should consider that food would bind the copper ions, preventing gastrointestinal symptoms. Thus consumption of the plain water and not copper with food would have a higher risk of triggering acute gastrointestinal manifestations. This is supported by a recent study in which we compared the incidence of nausea in subjects receiving beverages with graded copper concentration as plain water or as an orange-flavored drink (22). The incidence of nausea dropped from 54% (while taking copper in plain water) to 18% when subjects received equivalent copper concentrations in the orange-flavored beverage.

Our current study design does not permit a detailed examination of similar variables, such as consuming the water in the form of coffee or other forms where the copper may be absorbed in a third element (e.g., coffee grounds). Our study is also limited in that we did not control for the timing of water consumption. That is, we did not ask the subjects to record what times they consumed the

**Table 2.** Effect of copper on incidence of gastrointestinal symptoms (episodes per week in 45 women).

<table>
<thead>
<tr>
<th>Copper sulfate: copper oxide (mg/L)</th>
<th>0.5</th>
<th>1.4</th>
<th>2.3</th>
<th>3.2</th>
<th>5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea, vomiting, and abdominal pain</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea and vomiting</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea and abdominal pain</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Diarrhea only</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting and abdominal pain</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Abdominal pain only</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting only</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nausea only</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Episodes of diarrhea</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Gastrointestinal symptoms*</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>

*Diarrhea is not included.

**Table 3.** Gastrointestinal (GI) morbidity (mean episodes per subject per week) by study periods in 45 subjects.

<table>
<thead>
<tr>
<th>Morbidity</th>
<th>Periods with Cu added to water</th>
<th>Without periods, no Cu added</th>
<th>Wilcoxon matched pairs test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea (range)</td>
<td>0.04 (0.0–0.20)</td>
<td>0.01 (0.0–0.25)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>GI symptoms (range)</td>
<td>0.08 (0.0–0.60)</td>
<td>0.02 (0.0–0.25)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Total of GI symptoms (range)</td>
<td>0.12 (0.0–0.60)</td>
<td>0.03 (0.0–0.25)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
water nor if they consumed the water alone or with a meal. It is possible that subjects may have consumed the water with meals consciously or subconsciously to reduce gastrointestinal effects.

The aim of this study was to determine whether total copper concentration or the proportion of soluble copper was associated with gastrointestinal symptoms. We conclude that both copper sulfate (a soluble compound) and copper oxide (an insoluble compound) have comparable effects, implying that the ionic copper present in the stomach is responsible for the induction of gastrointestinal manifestations. People with achloridia or the elderly may be at increased risk for copper deficiency, as is the case for subjects consuming antacids, because copper absorption requires that the metal be in the ionized form. Further research is also needed to elucidate if less soluble compounds such as malachite \([\text{Cu}_2(\text{OH})_2\text{CO}_3]\) found in drinking water can also elicit gastrointestinal symptoms.

**References and Notes**