Acute Gastrointestinal Effects of Graded Levels of Copper in Drinking Water

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The objective of this study was to determine the acute gastrointestinal effects caused by the consumption of drinking water containing graded levels of added copper. Sixty healthy, adult women were randomly assigned to receive copper [Cu(II)] at four concentrations in their drinking water following a Latin-square design. Each group (n = 15) received tap water with no added copper, 1, 3, and 5 mg Cu/l of added copper sulfate for a 2-week study period, followed by 1 week of standard tap water. The subjects recorded their water consumption and gastrointestinal symptoms daily on a special form. The average daily consumption of water was 1.64 liters per subject, regardless of the amount of copper added. Final serum copper, ceruloplasmin, and liver enzymes were measured in all subjects and were not different from baseline concentrations. Twenty-one subjects (35%) recorded gastrointestinal disturbances sometime during the study, 9 had diarrhea, some with abdominal pain and vomiting, and 12 subjects presented abdominal pain, nausea, or vomiting. There was no association between copper levels in drinking water and diarrhea. However, nausea, abdominal pain, or vomiting were significantly related to copper concentrations in water. The recorded incidence rate of these symptoms was 5, 2, 17, and 15% while ingesting water with 0, 1, 3, and 5 mg Cu/l, respectively (overall χ^2 = 11.3, p<0.01; Cu ≤1 mg/l versus Cu ≥3 mg/l, χ^2, p<0.01). When subjects interrupted their consumption of drinking water with added copper, most symptoms disappeared. We conclude that under the conditions of the study, there was no association between aggregate copper in drinking water within the range of 0–5 mg/l and diarrhea, but a ≥3 mg Cu/l level of ionized copper was associated with nausea, abdominal pain, or vomiting. Additional studies with sufficient numbers of subjects are needed to define thresholds for specific gastrointestinal symptoms with precision and to extrapolate these results to the population at large. *Key words*: copper, diarrhea, drinking water, gastrointestinal symptoms. *Environ Health Perspect* 107:117–121 (1999). [Online 12 January 1999]
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Exposure to copper results almost exclusively from food and water intake, although small fractions come via inhalation and via skin contact with copper-containing substances (*1–7*). Intakes of copper at doses that exceed physiological requirements are normally controlled by efficient homeostatic mechanisms (*8,9*). Acute copper toxicity is infrequent in humans and is usually the consequence of consumption of contaminated foodstuffs or beverages, including drinking water, or from accidental or deliberate ingestion of high quantities of copper salts. Acute symptoms include excessive salivation, epigastric pain, nausea, vomiting, and diarrhea (*10–12*). Intravascular hemolytic anemia, acute hepatic failure, acute tubular renal failure, shock, coma, and death have been observed in severe copper poisoning (*5,7*). Anecdotal reports from isolated occurrences suggest that the consumption of beverages or drinking water contaminated with copper results in abdominal pain, nausea, vomiting, and diarrhea in humans. However, the threshold for gastrointestinal symptoms of copper in drinking water has not been precisely established (*13*) in controlled prospective studies. Furthermore, most studies have serious methodological problems, such as uncertainty of copper content in drinking water or confounding variables such as waterborne pathogens or alcohol intake. Demonstration of a causal relationship between copper ingestion and gastrointestinal symptoms has therefore been elusive. The aim of this work was to study the gastrointestinal effect induced by exposure to drinking water with graded levels of added soluble copper [Cu (II)].

Materials and Methods

This study was double-blind for the subjects and prospective in nature. Sixty healthy adult women were randomized into four copper groups, ingesting 0, 1, 3, and 5 mg of added ionic copper (as $CuSO_4 \cdot 5H_2O$) per liter of tap water. The corresponding concentrations of sulfate anion were 0, 1.5, 4.5, and 7.6 mg/l. Total duration of the study was 11 weeks, which were divided into four 2-week study periods, with 1 week rest in between. The average major ion concentrations in drinking water of the city of Santiago are 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 (0.03 mg/l). The subjects were of low socioeconomic status and lived in urban Santiago in a neighborhood constructed 13 years ago. All houses were of similar design and had a copper piping system. Copper content and pH of drinking water was measured using the EPA-220.1 method after 1 min at first-draw tap water. The women who participated in the study were healthy adults, did not work outside of the home, and were not pregnant or lactating.

All subjects were informed of the details of the project. Those who decided to participate signed a written consent form prior to their inclusion 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.

The 60 women were assigned to four groups of 15 subjects each, and the order of copper concentration tested followed a Latin-square design (Fig. 1), where A = .0 mg Cu/l; B = 1 mg Cu/l; C = 3 mg Cu/l, and D = 5 mg Cu/l. Thus, every woman served as her own control because each eventually experienced condition "A." There were no significant differences in general characteristics of the subjects among the groups (Table 1). The double-blinded nature of the study assured us that subjects did not know exactly what level of copper they were receiving. The Latin-square experimental design was used to control for potential sequence effects and confounding variables such as menstrual period, alcohol consumption, and food intolerance.

Every week the subjects received seven dark polypropylene flasks filled with a solution to add to their drinking water. Each participant prepared her water every morning by mixing the solution with tap water in a graduated 3-liter polypropylene container to produce the intended copper concentration upon mixing. At the end of day, the subject registered on a form the amount of water ingested and any gastrointestinal symptoms. That is, each woman recorded daily her symptoms, including nausea, vomiting, abdominal pain or cramps, diarrhea, and food intolerance. The subjects also recorded if they suffered from headache

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or if they were menstruating at the time. At the end of the day the subjects discarded the remaining noningested copper water.

A field dietitian visited each home twice each week to monitor preparation of drinking water with copper, to assure compliance of consumption of copper water, and to confirm recorded morbidity. If a subject presented diarrhea, abdominal pain, or vomiting, she was told not to ingest the water containing copper for the next 2 days. If the symptoms disappeared, the subject resumed consumption of the copper drinking water. If the symptoms reappeared during in the same study period, the subject was instructed not to drink the study water until the next 2-week study period began. Once each week verification of copper water concentration was performed by measuring the content of copper in a sample taken from the water prepared by each of the subjects. Copper provided by food was considered in the calculation of copper intake. On two occasions, the field dietitian obtained from each subject a 24-hr recall dietary questionnaire (*14*).

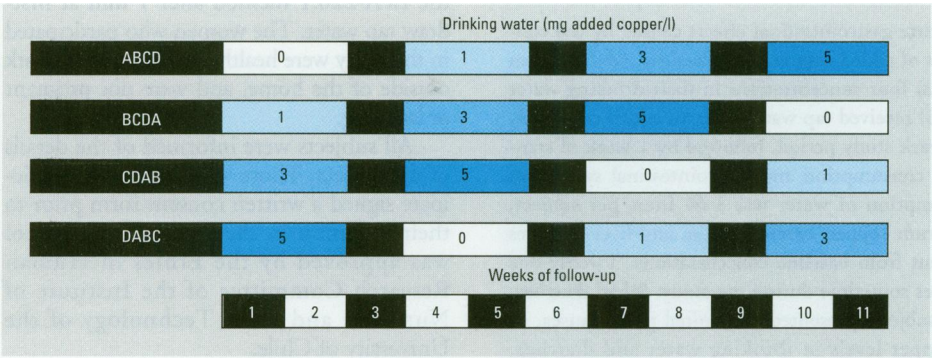

Figure 1. Experimental design of study. Sixty women were assigned to four groups of 15 subjects each with copper concentration patterns of 0, 1, 3, 5 (ABCD); 1, 3, 5, 0 (BCDA); 3, 5, 0, 1 (CDAB); and 5, 0, 1, 3 (DABC) mg Cu(II)/l.

Table 1. Characteristics of subjects

	Sequence of copper concentrations by groups			
	ABCD	BCDA	CDAB	DABC
n	15	15	15	15
Age (years)	33.1 ± 9.0	36.3 ± 11.0	32.3 ± 11.9	32.9 ± 10.8
Weight (kg)	62.3 ± 10.4	63.3 ± 13.5	64.9 ± 10.9	63.9 ± 16.8
Height (cm)	157 ± 5	155 ± 6	156 ± 3	158 ± 8
Copper IUD* (*n*)	6	5	4	6

Abbreviations: A, no added copper; B, 1 mg Cu (II)/l; C, 3 mg Cu (II)/l; D, 5 mg Cu (II)/l; IUD, intrauterine device.

Table 2. Effect of copper in drinking water on copper nutrition status and liver enzymes (geometric mean ± 1 standard deviation or range)

	Study groups				
	ABCD	BCDA	CDAB	DABC	Total
Hemoglobin (g/l)					
Baseline	134 ± 11	141 ± 08	134 ± 13	132 ± 10	135 ± 12
End first period	137 ± 12	142 ± 09	136 ± 16	133 ± 10	137 ± 13
End study	137 ± 14	143 ± 08	136 ± 16	133 ± 09	137 ± 13
Serum copper (µg/dl)					
Baseline	110 ± 21	112 ± 27	107 ± 20	104 ± 27	108 ± 24
End first period	112 ± 22	116 ± 19	116 ± 19	114 ± 23	115 ± 21
End study	111 ± 28	117 ± 32	117 ± 34	106 ± 34	113 ± 32
Ceruloplasmin (mg/l)					
Baseline	364 ± 82	421 ± 155	355 ± 66	364 ± 76	376 ± 105
End first period	356 ± 93	394 ± 108	360 ± 102	344 ± 84	363 ± 99
End study	390 ± 75	438 ± 124	392 ± 88	327 ± 58	387 ± 98
GGT (U/l)					
Baseline	12.3 (5.0–30.4)	12.0 (6.7–21.5)	13.1 (8.0–21.4)	7.8 (4.7–12.9)	11.1 (5.7–21.8)
End first period	8.8 (2.9–26.5)	8.9 (3.7–21.8)	9.3 (4.6–19.0)	6.6 (4.0–10.9)	8.4 (3.6–19.4)
End study	6.2 (1.8–21.0)	6.9 (3.1–15.1)	6.9 (2.8–16.9)	3.5 (2.0–6.3)	5.7 (2.2–14.6)
ALAT (U/L)					
Baseline	9.3 (3.9–22.3)	10.6 (5.3–21.2)	8.0 (2.8–23.2)	8.1 (3.4–19.4)	8.9 (3.7–21.8)
End first period	9.7 (4.9–19.1)	8.4 (3.2–21.9)	8.4 (3.9–18.2)	9.4 (6.3–14.2)	9.0 (4.3–18.7)
End study	6.3 (2.4–16.1)	7.8 (3.6–16.9)	9.3 (4.1–21.4)	6.7 (2.8–15.9)	7.4 (3.1–17.7)
AAT (U/l)					
Baseline	10.3 (4.1–25.5)	8.9 (4.0–19.6)	8.0 (2.5–26.0)	7.9 (3.9–15.8)	8.7 (3.5–21.8)
End first period	6.2 (2.4–15.6)	10.2 (5.9–17.6)	6.9 (2.7–17.9)	5.8 (2.8–11.9)	7.1 (3.1–16.3)
End study	8.7 (4.4–17.3)	6.3 (2.8–14.5)	10.6 (5.5–20.1)	6.7 (3.2–14.1)	7.9 (3.7–16.9)

Abbreviations: A, no added copper; B, 1 mg Cu (II)/l; C, 3 mg Cu (II)/l; D, 5 mg Cu (II)/l; GGT, L-γ-glutamyltransferase; ALAT, alanine-aminotransferase; AAT, aspartate-aminotransferase.

Blood samples were obtained 1 week before the study began, on the final day of the first 2 weeks, and on the final day at the end of the study to determine copper nutrition status. Serum copper was measured by atomic absorption spectrophotometry, ceruloplasmin by radial immunodiffusion (The Binding Site, Birmingham, UK), and aspartate-aminotransferase (AAT), alanine-aminotransferase (ALAT), L-γ-glutamyltransferase (GGT) by enzymatic assays (Boehringer, Mannheim, Germany); hemoglobin (Hb) was also measured (Coulter Electronics Inc., Hialeah, FL).

Geometric means for AAT, ALAT, and GGT were calculated because their distributions were non-Gaussians. Statistical analyses were carried out by analysis of variance (ANOVA), Chi-square tests, or Fisher's Exact tests.

Results

Serum copper levels at baseline were found to be within the normal range for women (65–185 µg/dl). The analyzed liver enzymes in all subjects were also within normal ranges. The levels of serum copper, ceruloplasmin, AAT, ALAT, and GGT remained within normal limits of the baseline both at the end of first period and at the end of the study (Table 2). There were no statistically significant differences in any of the parameters studied when the combined effect of time and group was analyzed by repeated-measures ANOVA: Hb ($F = 0.23$), serum copper ($F = 0.34$), ceruloplasmin ($F = 1.48$), AAT ($F = 1.29$), ALAT ($F = 0.65$), and GGT ($F = 0.53$). Repeated copper intakes from diet measured by 24-hr recall were 1.5 ± 0.4, 1.7 ± 0.5, 1.8 ± 0.3, and 1.9 ± 0.5 mg/day for groups ABCD, BCDA, CDAB, and DABC, respectively [ANOVA, not significant (NS)]. No changes were observed in body weight. The pH of standard tap water ranged from 7.3 to 7.8. At this pH range, 99.5% of Cu (II) is dissolved. The copper content of tap water was found to be below 0.1 mg/l and therefore was not considered to be a significant source of copper.

Study water consumption was similar among groups (Table 3). Seventy percent of the subjects consumed more than 1.5 liters daily, and only 3% recorded intakes below 0.5 liters water/day. Many of the subjects (27%) consumed the study water without any additives (such as powdered juice mix), 23% consumed it as tea, and 50% consumed it as tea, with a powdered juice mix, or without additives.

The mean measured concentrations of copper in drinking water prepared by the study subjects were 0.02 ± 0.01, 0.89 ± 0.25, 2.87 ± 0.45, and 4.57 ± 0.43 mg Cu/l for water expected to contain 0, 1, 3 and 5 mg Cu (II)/l, respectively. The subjects' total daily intakes of copper from water were 0.04 ± 0.02, 1.74 ± 0.66, 4.68 ± 2.24, and 7.94 ± 2.69 mg, respectively.

Table 4 shows a summary of the specific observed symptomatology by levels of copper in drinking water. Twenty-one subjects recorded gastrointestinal disturbances sometime during the study, 9 reported diarrhea with or without abdominal pain and vomiting, and the other 12 subjects presented abdominal pain, nausea, or vomiting. There was no association between copper levels in drinking water and diarrhea in the range of 0–5 mg/l, with or without vomiting and abdominal pain symptoms (χ^2, NS). None of the subjects presented episodes of diarrhea when ingesting water without the addition of copper. Four episodes of diarrhea occurred at concentrations of 1, 3, and 5 mg Cu/l each. On the other hand, 3, 1, 10, and 9 episodes of abdominal pain and/or nausea and/or vomiting were recorded when the subjects ingested water with 0, 1, 3 and 5 mg Cu/l, respectively (overall χ^2 = 11.3, p<0.01; Cu ≤1 mg/l versus Cu ≥3 mg/l, p<0.01). No differences were observed between 0 and 1 mg Cu/l, nor between 3 and 5 mg Cu/l. On discontinuing consumption of copper water, the symptoms persisted in subjects ingesting 0 mg Cu/l and disappeared in all subjects consuming drinking water with copper added. Symptoms reappeared in 23 of 27 episodes while subjects consumed drinking water with 3 and 5 mg Cu/l. Nongastrointestinal symptoms included seven subjects with headaches and six with increased salivation when they ingested water with 3 and 5 mg Cu/l; in contrast, three and two subjects exhibited these symptoms when they ingested water with 0 and 1 mg Cu/l, respectively (Fisher's Exact test, NS).

Figure 2 shows the gastrointestinal morbidity over time, independent of copper concentration in water, expressed as the ratio of episodes in each of the weeks relative to episodes occurring in the first week. Six of the 12 episodes of diarrhea that occurred throughout the study were presented during the first week of study; afterwards a significant decline was noted (χ^2 =19.1, p<0.008). The response followed a polynomial function as noted in Figure 2, suggesting that tolerance was induced over time. In contrast, the time distribution of abdominal pain, nausea, and vomiting was consistent throughout the study (seven in

Table 3. Mean group of study water intake (l/day)

Sequence groups	Time of study			
	1–2 weeks	4–5 weeks	7–8 weeks	10–11 weeks
ABCD	1.90 ± 0.69	1.73 ± 0.60	1.63 ± 0.64	1.61 ± 0.57
BCDA	1.79 ± 0.55	1.58 ± 0.73	1.39 ± 0.77	1.28 ± 0.91
CDAB	1.99 ± 0.61	1.85 ± 0.50	1.74 ± 0.68	1.71 ± 0.67
DABC	1.57 ± 0.53	1.52 ± 0.59	1.40 ± 0.56	1.51 ± 0.52
Total	1.81 ± 0.60	1.67 ± 0.61	1.54 ± 0.67	1.52 ± 0.69

Abbreviations: A, no added copper; B, 1 mg Cu (II)/l; C, 3 mg Cu (II)/l; D, 5 mg Cu (II)/l.

Table 4. Gastrointestinal (GI) and other symptoms according to copper concentration in drinking water

Reported symtoms	Drinking water (mg added Cu/l)			
	0 (n = 60)	1 (n = 60)	3 (n = 60)	5 (n = 60)
Diarrhea, vomiting, and abdominal pain	0	1	0	0
Diarrhea and vomiting	0	1	0	1
Diarrhea and abdominal pain	0	0	4	0
Diarrhea only	0	2	0	3
Abdominal pain only	2	1	3	2
Vomiting only	0	0	1	2
Nausea only	1	0	6	5
Total GI symptoms	3	5	14	13
Headache	2	1	3	4
Increased salivation	1	1	3	3
Total other symptoms	3	2	6	7

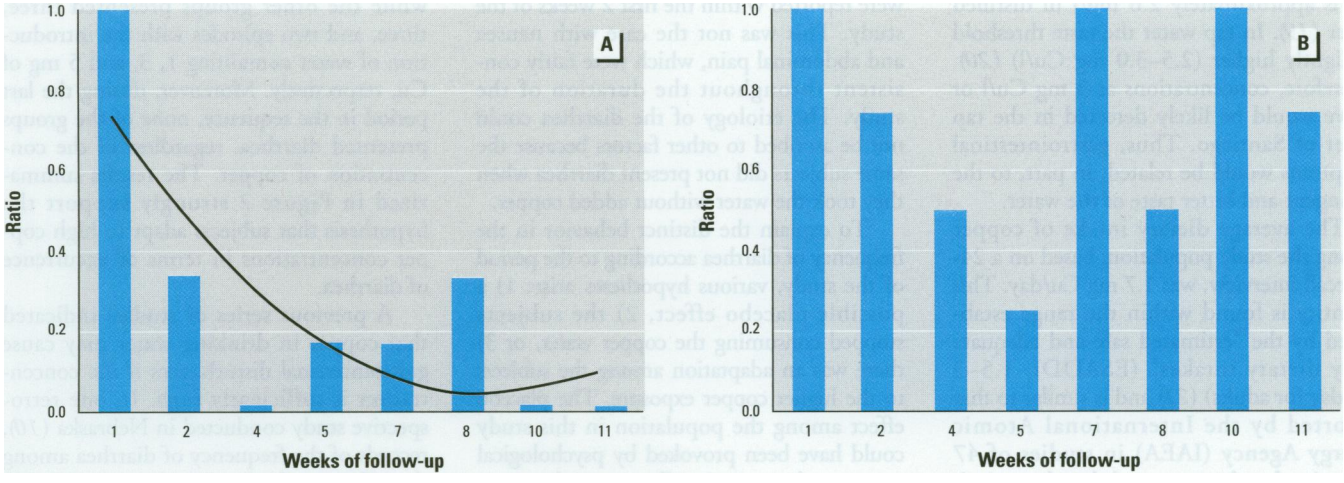

Figure 2. Gastrointestinal morbidity by week of study. (A) Ratio of episodes of diarrhea per week/episodes in the first week (χ^2, p<0.008) and binomial regression ($y = 95.02 - 23.25x + 1.46x^2$). (B) Ratio of episodes of nausea, abdominal pain, and vomiting per week/episodes in the first week (χ^2, not significant).

the first period, three in the second, six in the third, and seven in the last one; χ^2 = 2.06, NS).

Discussion

Copper ingested from food and water is almost never harmful to human health because the concentrations are low. Moreover, humans have mechanisms that maintain an efficient homeostasis of copper through a regulation of the absorption and the excretion of this micronutrient (i.e., when intake is high, absorption is low, and vice versa) (*15*). In this study, although the subjects ingested >200 mg Cu beyond their estimated needs during the 11 weeks of the study, no changes in the levels of serum copper, ceruloplasmin, and liver enzymes were noted. Based on available information on the effect of copper intake on absorption, we can speculate that most of the extra copper was not absorbed. This is supported by Turnlund et al. (*15*), who showed a copper absorption of 55, 36, and 12% in subjects receiving a low-copper diet (0.8 mg Cu/day), an adequate-copper diet (1.7 mg Cu/day), and a high-copper diet (7.5 mg Cu/day), respectively. Furthermore, a large proportion of the absorbed copper is excreted via the bile.

An excess in copper intake occasionally induces diarrhea and gastrointestinal upsets when ingested acutely (*10–12*). Hepatic damage has been reported when copper is ingested chronically at extremely high intake, >30 mg/day (*16–18*). In our study, the average ingestion of 1.8 liters of water with 5 mg of Cu/l [8.2 mg Cu(II)/day] for the first 14 days did not produce changes in liver enzymes. Throughout the study, the three liver enzymes measured remained stable and within normal ranges. These results were expected due to the short duration of the study and levels of copper intake.

The mean taste threshold for ionic copper is approximately 2.6 mg/l in distilled water (*19*). In tap water the taste threshold is slightly higher (2.5–3.0 mg Cu/l) (*20*). Therefore, concentrations at 3 mg Cu/l or above would be likely detected in the tap water of Santiago. Thus, gastrointestinal symptoms would be related, in part, to the astringent and bitter taste of the water.

The average dietary intake of copper among the study population, based on a 24-hr recall interview, was 1.7 mg Cu/day. This quantity is found within the range established by the "estimated safe and adequate daily dietary intakes" (ESADDI; 1.5–3 mg/day for adults) (*21*) and is similar to that reported by the International Atomic Energy Agency (IAEA) in studies of 47 countries that demonstrated that the majority of the countries have an average dietary intake of copper close to 1.5 mg/day (*22*).

Tap water was not a significant source of dietary copper, as the natural concentration of this micronutrient was only 0.02 mg/l. As expected, the overall intake of copper increased when the subjects were introduced to water with added copper. When consuming water with 1 mg Cu/l, the subjects received 3.5 mg Cu/day (1.8 mg of Cu from the water and 1.7 mg Cu from their diet). This is higher than the upper value of the ESADDI. With the level of 3 mg Cu/l in water, total copper consumption was 7.1 mg/day, which is almost double the ESADDI. At a level of 5 mg Cu/l, the total consumption of copper was 9.8 mg/day, approximately three times the upper value of ESADDI. However, all intake levels were below the limit of 0.5 mg of copper/kg body weight per day defined as precautionary intake level (maximum tolerable daily copper intake) by the Joint Food and Agricultural Organization of the United Nations/World Health Organization (WHO) Expert Committee on Food Additives (*23*) in the absence of an NOAEL (no-observed-adverse-effect level). Thus, our study indicates that acute reversible gastrointestinal symptoms occur below the limit provisionally established as safe in terms of chronic effects.

The results of our study show that there is no association between copper levels in drinking water and diarrhea with or without vomiting or abdominal pain in the range of 0–5 mg/l. At 3 mg Cu/l of water, a significant increase in reported nausea, abdominal pain, or vomiting occurs. Close to a third of the subjects presented mild gastrointestinal disturbances during the study. Nine women (15%) presented 12 episodes of diarrhea, and the remaining 12 (20%) suffered from nausea, abdominal pain, or vomiting. It is important to recognize that 8 of the 12 episodes of diarrhea were reported within the first 2 weeks of the study. This was not the case with nausea and abdominal pain, which were fairly consistent throughout the duration of the study. The etiology of the diarrhea could not be ascribed to other factors because the same subjects did not present diarrhea when they took the water without added copper.

To explain the distinct behavior in the frequency of diarrhea according to the period of the study, various hypotheses arise: 1) a possible placebo effect, 2) the subjects stopped consuming the copper water, or 3) there was an adaptation among the subjects to the higher copper exposure. The placebo effect among the population in this study could have been provoked by psychological concerns that occasionally occur when participating in experimental studies. However, it is not likely that a placebo effect can explain the diarrhea because the frequency of other symptoms such as abdominal pain, nausea, and vomiting had a different distribution. The fact that the group consuming water without added copper presented only two episodes of gastrointestinal symptomatology does not support the placebo hypothesis either. In fact, the frequency of gastrointestinal symptoms observed in this study is comparable to the results of a previous study in Santiago indicating that apparently healthy women self-report 0.22 gastrointestinal episodes per subject per year (*24*). Our rate is slightly higher (0.54), and this could be explained by the fact that subjects in this study were asked to report symptoms and thus may have had a greater sensitivity to symptoms. We purposely asked them to record any discomfort they felt. Compliance was monitored, but we could not be certain that they prepared and consumed the water as instructed. Although the population studied was highly motivated to fulfill their obligation to drink the copper water as instructed, it must be noted that in all groups participant consumption of the experimental water decreased from 1.81 liters in weeks 1 and 2 to 1.52 liters in weeks 11 and 12.

By reducing the water consumption, subjects reduced their copper exposure and subsequent risk of developing gastrointestinal upsets. This further suggests that self-report of consumption may be overestimated. The additional verification of copper levels in water, performed by measuring the content of copper in the water prepared in the containers by the subjects demonstrated that the subjects prepared the copper water correctly.

The possible adaptation to rising copper concentration was best exemplified in the ABCD group, which received 0, 1, 3, and 5 mg Cu/l. This was the only group that did not present episodes of diarrhea, while the other groups presented three, three, and two episodes with the introduction of water containing 1, 3, and 5 mg of Cu, respectively. Moreover, during the last period in the sequence, none of the groups presented diarrhea, regardless of the concentration of copper. The results summarized in Figure 2 strongly support the hypothesis that subjects adapt to high copper concentrations in terms of occurrence of diarrhea.

A previous series of studies indicated that copper in drinking water may cause gastrointestinal disturbances if the concentration is sufficiently high. In one retrospective study conducted in Nebraska (*10*), records of the frequency of diarrhea among the subjects who lived in houses with different concentrations of copper in potable water (<1.3 mg/l, 2–3 mg/l, and >3 mg/l)

were obtained. Subjects that lived in houses with levels of copper >3 mg/l of water presented a higher frequency of diarrhea than the other groups. It was concluded that a high risk of developing diarrhea exists for people ingesting water with copper content >3 mg/l. Later, within this same population, the copper content of the water in the homes of the subjects who presented diarrhea and in homes of healthy subjects was compared, but no differences were found. This second study invalidated the conclusions established previously. The discrepancy was attributed to the fact that the water samples in the second study were collected 3 weeks after the report of the illness, while in the first study the samples were collected 7 months after the manifestation of illness.

Two other published studies (*11,12*) have serious methodological problems, such as not taking certain factors into account, and so they do not permit a definitive statement to be made about the gastrointestinal effects of high concentrations of copper in tap water. In our study we controlled for factors such as diet, alcohol consumption, and presence of menstruation, and because randomization included a no-copper-added group, we could control for confounders. In our study, 25% of the subjects presented gastrointestinal symptoms when they abruptly changed the consumption of potable water from their homes (0.02 mg Cu/l) for water with 1 mg Cu/l or more. However, the presence of diarrhea tended to disappear over time. No association between copper concentration and incidence of diarrhea was observed.

The limit of 2 mg Cu/l, the provisional guideline value for copper in drinking water suggested by the World Health Organization as safe for human consumption, is below the 3 mg/l that we tested. Another controlled study in infants carried out by our group demonstrated that the ingestion of water with 2 mg Cu(II)/l did not produce more episodes of diarrhea than the consumption of water with <0.1 mg Cu(II)/l (*25*). Clearly, this study only deals with symptomatology in adults, but infants and children may perhaps be affected to a greater degree, as suggested in other studies (*12*). Therefore, further investigation in this area is warranted so that the appropriate and safe levels for children can be determined.

In conclusion, our study demonstrates that copper in drinking water, as ionized copper, showed no association between copper levels and diarrhea, although at concentrations ≥3 mg/l copper is associated with an increase in gastrointestinal symptoms (nausea, abdominal pain, or vomiting) above baseline. This level of ionic copper in drinking water may be associated with higher total copper content, as several copper species found in drinking water are not fully soluble. Thus, drinking water with higher copper concentration may be well tolerated. Further studies are required to determine the population-based copper concentration threshold for different gastrointestinal symptoms and the specific response to copper species actually found in drinking water.

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