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Associations of Dietary and Serum Copper with Inflammation, Oxidative Stress, and Metabolic Variables in Adults[1,2]

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Abstract

There are conflicting data on the associations between copper and glycemia, plasma lipids, and atherosclerotic diseases. Copper has both pro-oxidant and antioxidant effects. We performed a cross-sectional analysis to investigate the associations between dietary copper intake and metabolic variables and serum high-sensitivity C-reactive protein (hs-CRP) in asymptomatic subjects from a population-based cohort ($n = 1197$) and between serum copper concentration and markers of oxidative stress, including plasma nitrotyrosine (NT) and total antioxidant status (TAS), hs-CRP, and metabolic variables in a subgroup of men from this cohort ($n = 231$). In all subjects, diastolic blood pressure and circulating glucose, uric acid, and total and LDL-cholesterol concentrations significantly decreased, whereas the hs-CRP concentration increased, from the lowest to the highest tertile of copper intake. In the male subgroup, glucose and total and LDL-cholesterol and TAS decreased, whereas hs-CRP and NT concentrations increased from the lowest to the highest tertile of serum copper concentration. In multiple regression models, dietary copper intake was inversely associated with diastolic blood pressure ($P = 0.002$), fasting glucose ($P < 0.001$), total cholesterol ($P < 0.001$), LDL-cholesterol ($P < 0.001$), and uric acid ($P < 0.001$) and was directly associated with the hs-CRP concentration ($P < 0.001$). Serum copper concentrations were inversely associated with glucose ($P < 0.001$), total cholesterol ($P < 0.001$), LDL-cholesterol ($P < 0.001$), and TAS ($P < 0.001$) and were directly associated with hs-CRP ($P < 0.001$) and NT concentrations ($P < 0.001$). Marginal copper deficiency is associated with an unfavorable metabolic pattern, but copper supplementation might not be recommended in view of its association with inflammation and markers of oxidative stress. J. Nutr. 138: 305–310, 2008.

Introduction

Copper is an essential nutrient in humans and acts as a critical cofactor when incorporated into specific cupro-enzymes that catalyze electron transfer reactions required for cellular respiration, iron oxidation, pigment formation, neurotransmitter biosynthesis, antioxidant defense, peptide amidation, and connective tissue formation (1). Both overt copper deficiency and excess are associated with specific clinical manifestations (1,2). Copper deficiency causes an accumulation of arterial lipid peroxides, possibly due to the decreased activity of the copper-zinc superoxide dismutase (CuZnSOD)[6]. Many of the pathological effects of copper overload are consistent, however, with oxidative damage to membranes or macromolecules (3). Copper intake in vivo has shown both pro-oxidant and antioxidant effects; ceruloplasmin, the major copper-containing plasma protein, may act as either an antioxidant or pro-oxidant, depending on ambient conditions (3). Thus, the definitive role of copper in oxidative processes is still a matter of debate. Furthermore, experimental and epidemiological data regarding the possible role of copper on metabolic abnormalities and atherogenesis are conflicting.

Increased concentrations of plasma copper were observed in diabetic patients with chronic complications or macrovascular diseases (4). On the other hand, copper supplementation exerted beneficial effects in diabetic rats by reducing glucose levels (5).

Both inverse and direct correlations between serum and dietary copper and total cholesterol concentrations have been reported (3,6).

The zinc/copper hypothesis proposed an increased atherosclerotic risk in copper deficiency due to hypercholesterolemia (7). In contrast, epidemiological studies showed that higher serum copper concentrations may promote coronary artery diseases (8,9).

In this study, we performed a cross-sectional analysis to investigate the associations between dietary copper intake with

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[6] Abbreviations used: CuZnSOD, copper-zinc superoxide dismutase; hs-CRP, high-sensitivity C-reactive protein; NT, nitrotyrosine; SFFQ, semiquantitative FFQ; TAS, total antioxidant status.
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metabolic variables and high-sensitivity C-reactive protein (hs-CRP) in apparently healthy subjects from a population-based cohort and serum copper concentrations with markers of oxidative stress [nitrotyrosine (NT), total antioxidant status (TAS)] and inflammation and metabolic variables in a subgroup of men from this cohort.

Patients with known diseases and/or taking vitamin/micronutrient supplementation or drugs were excluded from the study to avoid the effects of a particular diet as a consequence of their pathologies and the effects of medication on copper metabolism.

Materials and Methods

We conducted a metabolic screening between 2001 and 2003 in a representative cohort of adults from Asti (northwestern Italy) (10). Briefly, we contacted all 45- to 64-y-old subjects ($n = 1877$) from 6 family physicians whose patients were representative of the local health districts. We screened 1658 subjects (88.3%) who gave their informed consent to participate. All procedures were in compliance with the Helsinki Declaration principles.

Both participants and nonparticipants were similar to the age-corresponding population for sex distribution, education level, prevalence of known diabetes, and percentage of subjects living in a rural area (10). All participants completed a FFQ and underwent several clinical and laboratory tests (11). Three hundred men from this population-based cohort were randomly identified (12); their TAS and NT concentrations were measured.

The exclusion criteria included known diseases requiring specific dietary recommendations (diabetes, cardiovascular diseases, dyslipidemia, chronic liver/bowel, or kidney disease) and subjects on vitamin/micronutrient supplementations or drugs (estrogens included).

The remaining apparently healthy subjects who reported none of the above conditions, 1197 of the 1658 subjects from the entire cohort and 231 out of the 300 men from the subsample, were evaluated.

Copper intake was evaluated in all subjects ($n = 1197$), whereas serum copper concentrations were measured in the randomly identified subgroup of men ($n = 231$).

The semiquantitative FFQ (SFFQ) used in the European Prospective Investigation into Cancer and Nutrition studies was used for all subjects. It assessed mean frequency and portion size of 148 foods consumed during the 12 mo prior to examination. Nutrient intake was calculated by multiplying the frequency of consumption of each food by the nutrient content of the specified portion size. The reproducibility and validity of the European Prospective Investigation into Cancer and Nutrition-SFFQ was previously validated (13).

Dietary copper intake was not assessed by this tool; its intake was computed by multiplying copper content of the specific serving of each food item [according to the tables of food composition of the Italian National Institute of Nutrition (14)] by the frequency of its daily consumption and summing all items.

Each nutrient was adjusted for total energy, using the residual method, according to the following formula: energy-adjusted nutrient intake = a + b, where a = the residual for each subject from the regression model with nutrient intake as the dependent variable and total energy intake as the independent variable, and b = the expected nutrient intake for a person with mean energy intake (15). Dietary energy-adjusted copper intake and serum concentrations were divided into tertiles.

After an overnight fast, we measured weight and waist circumference (at the level of the umbilicus) and took blood pressure for analysis of glucose, total cholesterol, HDL-cholesterol, triglyceride, insulin, uric acid, and hs-CRP concentrations. Systolic and diastolic blood pressures were measured twice with subjects in a sitting position after at least 10 min of rest using a standard mercury sphygmomanometer. Values reported are the means of the 2 determinations.

Insulin resistance was calculated from the homeostasis model assessment-insulin resistance model (HOMA-IR) according to the following formula: fasting insulin (pmol/L) × fasting glucose (mmol/L)/22.5 (16).

The level of physical activity during leisure was defined as light (inactive, <4 h/wk physical activity), moderate (4 h/wk), and heavy (regular, >4 h/wk) (11).

Laboratory methods for analyzing serum glucose, hs-CRP (10), insulin, uric acid, and prealbumin (11), and plasma total cholesterol, HDL-cholesterol, triglyceride (10,11), and NT (12) have been described. LDL-cholesterol was calculated using the Friedewald equation [LDL-cholesterol = total cholesterol – (HDL-cholesterol + triglycerides/5)].

Serum copper concentration was determined by flame atomic absorption spectrometry (5110 PC spectrometer Perkin-Elmer), which measured both bound and unbound serum copper with a CV of 3.6–4.9%. The evaluation of TAS levels in samples was based on the reduction of Cu^{++} into Cu^{+} by the action of all present antioxidants. The amount of Cu^{+} was determined by measuring the complex formed by Cu^{+} and bathocuproine. This complex has a typical absorption at 490 nm (ANTOXT kit by Fujirebio Diagnostics).

Statistical analyses

Values in the text are means ± SD, unless otherwise noted.

Because the distribution of hs-CRP, insulin, HOMA-IR score, and triglyceride concentrations was positively skewed, their values were log-transformed, thus approximating a normal distribution. The log-transformed values of these variables were then used in all analyses. For ease of interpretation, the median (and interquartile range) of nontransformed values are reported. Chi-square test was used to evaluate gender difference in the prevalence of subjects who consumed less than the recommended dietary copper intake.

Associations between clinical and laboratory variables (dependent variables) and tertiles of dietary copper intake or serum concentrations were evaluated by logistic regression analyses for discrete variables or linear regression for continuous variables. Post hoc analysis (Bonferroni's correction) was used to compare the tertiles.

Variables significantly associated with dietary or serum copper values were analyzed by multiple regression analyses after adjustments for multiple confounders (SAS, version 8.0; SAS Institute). Significant P-values were 2-sided at $\alpha < 0.05$.

Results

Median copper intake was 1.5 mg/d; 57% of males and 46% of females ($P < 0.001$) consumed less than the recommended amount of 1.5–3 mg/d (17).

The major sources of dietary copper were dairy products (24.6%), fruit (22.0%), bread (13.4%), fish (12.9%), pasta (11.8%), vegetables (5.5%), and legumes (5.5%).

Subjects from the entire cohort in the highest tertile of copper intake had significantly higher intakes of cholesterol, fiber, magnesium, vitamin C, vitamin E, and β-carotene and significantly lower intake of PUFA than subjects in the lowest tertile (**Table 1**).

In the entire cohort, diastolic blood pressure and circulating glucose, total cholesterol, LDL-cholesterol, and uric acid concentrations significantly decreased from the lowest to the highest tertile of copper intake, whereas the hs-CRP concentration increased (Table 1).

In the subgroup of men, TAS significantly decreased from the lowest to the highest tertile of copper dietary intake and the NT concentration increased (**Table 2**). Their serum copper concentration was 15.0 ± 7.1 μmol/L, similar to values reported in other populations (~16 μmol/L) (18). Dietary intake and serum concentrations of copper were correlated in this subgroup ($r = 0.56$; $P < 0.001$). Concentrations of glucose, total cholesterol and LDL-cholesterol, and TAS significantly decreased from the lowest to the highest tertile of serum copper, whereas hs-CRP and NT concentrations increased in these men (Table 2).

In all subjects, dietary intake of copper was inversely associated with diastolic blood pressure and fasting glucose, total cholesterol, LDL-cholesterol, and uric acid concentrations and was directly associated with the hs-CRP concentration in both simple and multiple regression models after adjustment for multiple confounders (**Table 3**).

TABLE 1 Clinical, dietary, and laboratory characteristics of adult men and women according to tertiles of copper intake[1,2]

	1st tertile (lowest)	2nd tertile	3rd tertile (highest)	P^3
n	399	399	399	
Copper intake, *mg/d*	1.12 (0.29)	1.48 (0.17)	2.29 (1.08)	
Males, *%*	47.4	47.4	47.4	
Smoker, *%*				
Former	23.6	21.8	24.6	
Current	22.3	22.8	22.1	0.84
Physical activity, *%*				
Inactive	39.1	40.8	42.6	
Active	60.9	59.2	57.4	0.31
Alcohol consumption, *%*				
Abstainers	43.9	42.6	44.1	
≥30 g/d	16.8	18.3	16.5	0.92
Dietary variables				
Total energy, *kJ/d*	8810.7 ± 2952.9	8265.5 ± 2310.7	9041.6 ± 2849.8	0.23
Cholesterol, *mg/d*	331.3 ± 87.6	330.0 ± 78.8	349.7 ± 88.0[b]	0.001
Total fat, *% energy*	35.3 ± 6.3	35.1 ± 5.5	35.6 ± 5.5	0.54
SFA, *% energy*	11.8 ± 2.7	11.7 ± 2.3	12.1 ± 2.6	0.08
PUFA, *% energy*	4.4 ± 1.5	4.2 ± 1.1	4.2 ± 1.2[a]	0.043
Carbohydrate, *% energy*	48.9 ± 7.7	48.6 ± 6.7	48.1 ± 6.9	0.10
Fiber, *g/d*	18.9 ± 8.3	19.2 ± 5.7	23.6 ± 8.8[c]	<0.001
Magnesium, *mg/d*	295.8 ± 83.6	306.6 ± 57.9[a]	358.9 ± 99.0[c]	<0.001
Zinc, *mg/d*	12.5 ± 2.6	12.1 ± 2.0	12.8 ± 2.4	0.06
Vitamin C, *mg/d*	120.1 ± 67.0	122.1 ± 54.3	150.5 ± 96.9[c]	<0.001
β-carotene, μ*g/d*	3661.0 ± 1664.9	3742.4 ± 1508.7	4359.1 ± 2263.3[c]	<0.001
Vitamin E, *mg/d*	8.0 ± 3.2	7.8 ± 2.4	9.1 ± 4.1[c]	<0.001
Clinical and laboratory data				
Age, *y*	54.2 ± 5.8	54.5 ± 5.6	54.8 ± 5.9	0.11
BMI, *kg/m*^{2}	26.4 ± 4.5	26.4 ± 4.2	27.0 ± 5.2	0.052
Waist circumference, *cm*	91.7 ± 13.4	91.0 ± 12.5	92.8 ± 12.7	0.23
Systolic blood pressure,[4] *mm Hg*	134.0 ± 15.6	134.1 ± 15.7	132.7 ± 15.7	0.23
Diastolic blood pressure,[4] *mm Hg*	84.0 ± 9.0	83.1 ± 9.5	82.4 ± 8.8[b]	0.014
Serum fasting glucose, *mmol/L*	5.9 ± 1.8	5.8 ± 1.5	5.4 ± 0.8[c]	<0.001
Plasma total cholesterol, *mmol/L*	5.8 ± 1.1	5.7 ± 1.1	5.4 ± 1.0[c]	<0.001
Plasma LDL-cholesterol, *mmol/L*	3.9 ± 1.0	3.8 ± 1.0	3.6 ± 0.9[c]	<0.001
Plasma triglycerides,[5] *mmol/L*	1.3 (0.9)	1.4 (0.9)	1.3 (0.8)	0.21
Plasma HDL-cholesterol, *mmol/L*	1.6 ± 0.4	1.6 ± 0.3	1.5 ± 0.3	0.13
HOMA-IR,[5] *mmol/L*×*pmol/L*	3.0 (5.5)	3.2 (6.9)	3.1 (5.5)	0.99
Serum hs-CRP,[5] *mg/L*	1.4 (2.4)	1.4 (2.3)	1.8 (2.6)[b]	0.005
Serum uric acid, *mmol/L*	204.3 ± 65.7	197.6 ± 59.0	192.7 ± 59.4[b]	0.008
Serum prealbumin, μ*mol/L*	459.0 ± 148.7	450.7 ± 145.0	464.6 ± 140.4	0.59

[1] Values are medians (interquartile range), means ± SD, or percent.
[2] Nutrient intakes were energy adjusted. Tertile 1 was the lowest copper intake and tertile 3 was the highest. Cutoffs for tertiles of copper intake were <1.24, 1.24–1.63, and >1.63 mg/d for men and <1.39, 1.39–1.75, and >1.75 mg/d for women.
[3] *P*-value was determined by linear regression or logistic regression. Superscript letters indicate a difference from the first tertile group within a row: [a]$P \leq 0.05$; [b]$P \leq 0.01$; [c]$P \leq 0.001$.
[4] Mean of 2 determinations.
[5] Nonnormally distributed variable.

In the subgroup of men, dietary intake and serum concentrations of copper were directly associated with NT and inversely associated with TAS (Table 3). In a multiple regression model, the serum copper concentration was inversely associated with concentrations of glucose, total cholesterol, and LDL-cholesterol and directly associated with hs-CRP.

The results did not change after we adjusted the data for dietary intake of carbohydrates or antioxidant vitamins or when we considered absolute rather than energy-adjusted copper intake. When we restricted the analyses to subjects with adequate copper intake ($n = 595$ in the entire cohort and 104 in the male subgroup), the results did not change significantly, with the exception of the associations between dietary copper and TAS values, which were no longer significant (data not shown).

Discussion

Approximately one-half of our population-based sample consumed less than the recommended daily copper intake (17). Consistent with this, an increasing number of patients with acquired copper deficiency have recently been described, suggesting that this problem might be more widespread than currently realized (1).

The amount of dietary copper strongly influences absorption: as the former increases, the fraction absorbed declines. This was

TABLE 2 Clinical and laboratory characteristics in the subgroup of men according to tertiles of copper intake and serum copper concentrations[1,2]

	1st tertile (lowest)	2nd tertile	3rd tertile (highest)	P^3
n	77	77	77	
Copper intake, *mg/d*	0.99 (0.41)	1.44 (0.20)	2.08 (1.03)	
Plasma NT,[4] *μmol/L*	3.9 (4.9)	5.3 (13.8)[a]	6.0 (26.2)[a]	0.009
TAS, *mmol/L*	0.52 ± 0.32[5]	0.42 ± 0.22[a]	0.40 ± 0.21[b]	0.005
Serum copper concentrations[5]				
Serum copper, *μmol/L*	12.0 ± 1.5	15.0 ± 0.6	18.2 ± 1.9	
Age, *y*	54.6 ± 5.3	54.0 ± 5.8	54.9 ± 5.4	0.71
BMI, *kg/m²*	26.7 ± 3.6	27.7 ± 4.8	27.7 ± 5.0	0.17
Waist circumference, *cm*	96.2 ± 12.9	97.9 ± 11.0	98.5 ± 12.7	0.24
Systolic blood pressure,[6] *mm Hg*	138.1 ± 14.9	134.2 ± 16.5	134.7 ± 15.9	0.19
Diastolic blood pressure,[6] *mm Hg*	86.1 ± 9.3	83.5 ± 9.1	84.7 ± 9.2	0.35
Serum fasting glucose, *mmol/L*	6.5 ± 2.2	6.3 ± 1.8	5.8 ± 1.2[b]	0.030
Plasma total cholesterol, *mmol/L*	5.9 ± 1.0	5.4 ± 1.1[a]	5.3 ± 0.9[c]	<0.001
Plasma LDL-cholesterol, *mmol/L*	4.0 ± 1.0	3.7 ± 1.1[a]	3.5 ± 1.0[c]	<0.001
Plasma triglycerides,[4] *mmol/L*	1.8 (1.0)	1.6 (0.8)	1.4 (1.1)	0.15
Plasma HDL-cholesterol, *mmol/L*	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	0.99
HOMA-IR,[4] *mmol/L×pmol/L*	3.4 (6.9)	4.7 (9.7)	3.3 (7.6)	0.61
Serum hs-CRP,[4] *mg/L*	0.8 (1.3)	1.4 (1.6)[a]	1.6 (2.9)[c]	<0.001
Serum uric acid, *mmol/L*	236.7 ± 69.5	226.2 ± 63.9	221.3 ± 61.2	0.14
Plasma NT,[4] *μmol/L*	3.6 (7.0)	4.8 (14.3)[b]	6.2 (24.9)[b]	<0.001
TAS, *mmol/L*	0.51 ± 0.31	0.46 ± 0.22	0.37 ± 0.21[c]	<0.001

[1] Values are median (interquartile range) or mean ± SD.
[2] Nutrient dietary intake is energy adjusted. Tertile 1 was the lowest copper intake and tertile 3 was the highest. Cutoffs for tertiles of copper intake were <1.25, 1.25–1.66, and >1.66 mg/d.
[3] *P*-value was determined by linear regression analysis. Superscript letters indicate a difference from the first tertile group within a row: [a]$P \leq 0.05$; [b]$P \leq 0.01$; [c]$P \leq 0.001$.
[4] Nonnormally distributed variable.
[5] Tertile 1 was the lowest copper serum values and tertile 3 was the highest. Cutoffs for tertiles of serum copper concentrations were <14.0, 14.0–15.9, and >15.9 μmol/L.
[6] Mean of 2 determinations.

reflected in the modest association between copper intake and the serum concentration in the subgroup of men ($r = 0.56$; $P < 0.001$).

In our subjects, both dietary and serum copper were inversely associated with fasting glucose, total cholesterol, and LDL-cholesterol after adjustment for multiple confounders. Dietary cholesterol intake was higher in subjects in the higher dietary copper tertiles, whereas their cholesterol concentrations were lower.

The direct relationships between dietary and serum copper with hs-CRP and NT and the inverse relationship with TAS suggest a possible participation of copper in inflammatory and pro-oxidant mechanisms, but this is unproven at this time.

Copper and metabolic abnormalities. Currently, data regarding dietary intake and blood concentrations of copper and glucose are conflicting. In rats, glycation was enhanced in dietary copper deficiency (19), whereas copper supplementation reduced glucose levels in diabetic mice (5); in rat isolated adipocytes, copper sulfate inhibited free fatty acid release and enhanced glucose uptake (20). Thus, the authors hypothesized an insulin-mimetic activity for copper.

Other studies found that in diabetic patients, circulating copper concentrations were not different (21) or were greater in patients with chronic complications or macrovascular diseases (4). Indeed, in the presence of overt diabetes with chronic complications, the associated chronic low-grade inflammatory state might be responsible for an increase in blood copper concentrations.

There are several reports of an inverse correlation between serum and dietary copper and total and LDL-cholesterol in experimental studies in humans and rats [reviewed in (3)]. In contrast, a positive association between serum copper concentrations and cholesterol has been reported (6).

The mechanisms by which copper deficiency may act on LDL-cholesterol may include: decreased heparin-releasable lipoprotein-lipase activity (22), increased clearance of cholesterol esters (with newly synthesized cholesterol esters entering the serum pool at an increased rate) (23), enhanced hepatic apolipoprotein B synthesis (24), and increased hydroxymethylglutaryl CoA reductase activity (25).

In copper-deficient rats, HDL-cholesterol was significantly increased, likely due to lower HDL-binding in the liver (26), whereas in humans, the increase in plasma lipids and apolipoproteins was associated mainly with LDL-cholesterol (27), consistent with our results.

A relationship between copper deficiency and hypertension was reported, perhaps from impaired vasodilatation in response to acetylcholine (28). We found an inverse association between diastolic blood pressure and dietary copper intake in the entire cohort but not for serum copper concentrations in the subsample of men, even though systolic and diastolic blood pressures were higher in subjects in the lowest serum copper tertile than in subjects in the other 2 tertiles (Table 2).

Copper and oxidative stress. Copper is both a pro-oxidant and an antioxidant. Its antioxidant activity has been attributed to increased CuZnSOD activity (3). Copper supplementation in healthy volunteers showed an antioxidant effect, because it protected RBC from oxidation (29). However, this effect did not result from increased CuZnSOD activity and was evident for supra-physiological doses of copper only (7 mg/d).

TABLE 3 Associations between the variables listed and dietary intake and serum copper concentrations in a multiple regression model[1,2]

		β (95%CI)	*P*
Dietary copper			
All subjects, $n = 1197$			
Diastolic blood pressure, *mm Hg*	Crude	−0.80 (−1.42, −0.18)	0.011
	Adjusted	−0.94 (−1.56, −0.32)	0.002
Serum fasting glucose, *mmol/L*	Crude	−0.29 (−0.39, −0.19)	<0.001
	Adjusted	−0.26 (−0.36, −0.16)	<0.001
Plasma total cholesterol, *mmol/L*	Crude	−0.23 (−0.30, −0.16)	<0.001
	Adjusted	−0.26 (−0.33, −0.19)	<0.001
Plasma LDL cholesterol, *mmol/L*	Crude	−0.21 (−0.27, −0.15)	<0.001
	Adjusted	−0.22 (−0.29, −0.15)	<0.001
Serum hs-CRP, *mg/L*	Crude	0.27 (0.20, 0.34)	<0.001
	Adjusted	0.36 (0.30, 0.42)	<0.001
Serum uric acid, *mmol/L*	Crude	−7.81 (−11.9, −3.67)	<0.001
	Adjusted	−6.66 (−10.6, −2.74)	<0.001
Subgroup of men, $n = 231$			
Plasma NT, μ*mol/L*	Crude	0.64, (0.37, 0.91)	<0.001
	Adjusted	0.65, (0.38, 0.92)	<0.001
TAS, *mmol/L*	Crude	−0.05, (−0.08, −0.02)	0.006
	Adjusted	−0.05, (−0.09, −0.01)	0.012
Serum copper			
Serum fasting glucose, *mmol/L*	Crude	−0.09, (−0.17, −0.01)	0.017
	Adjusted	−0.13, (−0.21, −0.05)	<0.001
Plasma total cholesterol, *mmol/L*	Crude	−0.10, (−0.14, −0.06)	<0.001
	Adjusted	−0.09 (−0.14, −0.04)	<0.001
Plasma LDL cholesterol, *mmol/L*	Crude	−0.09 (−0.14, −0.05)	<0.001
	Adjusted	−0.09 (−0.14, −0.05)	<0.001
Serum hs-CRP, *mg/L*	Crude	0.08 (0.04, 0.12)	<0.001
	Adjusted	0.07 (0.03, 0.12)	<0.001
Plasma NT, μ*mol/L*	Crude	0.16 (0.07, 0.25)	<0.001
	Adjusted	0.16 (0.07, 0.25)	<0.001
TAS, *mmol/L*	Crude	−0.02 (−0.03, −0.01)	<0.001
	Adjusted	−0.02 (−0.03, −0.006)	<0.001

[1] Regression analysis evaluating the linear relationships between dietary or serum copper and each laboratory variable (crude).
[2] Multiple regression analysis evaluating the association between dietary or serum copper and each laboratory variable after adjustment for age, BMI, exercise level, smoking, and dietary intake of total energy, cholesterol, magnesium, zinc, alcohol, and (in all subjects) sex (adjusted).

On the other hand, copper ions participate in radical reactions such as the conversion of superoxide to hydrogen peroxide and hydroxyl radicals, and catalyze the oxidative modification of LDL in vitro and in the arterial wall; copper excess can induce oxidative damage to DNA (2,3).

Increased concentrations of lipid peroxides were found in women using oral contraceptives; estrogen treatment resulted in increased plasma copper and there was a strong relationship between plasma copper and lipid peroxides in these patients ($r = 0.84$; $P < 0.001$) (30).

We found a significant association between markers of oxidative stress (higher NT and lower TAS) and increasing dietary and serum copper concentrations in the subsample of men, even for values within normal ranges (18).

Our results add to the controversy concerning the oxidant and antioxidant effects of copper. Many previous studies were performed in vitro or under conditions of copper deficiency or excess. In vivo, in our patients with blood copper concentrations within normal ranges, the pro-oxidant effect of copper seemed to predominate.

Copper and inflammation. Ceruloplasmin responds as an acute-phase reactive protein to stress and trauma and increased copper concentration was reported in response to inflammation, infection, and various chronic diseases, such as arthritis and neoplasia (31). Serum copper concentration is higher than normal in various inflammatory diseases in humans and laboratory animals (32). The rise of ceruloplasmin is probably responsible for the increased serum copper in the aforementioned conditions.

We found a significant direct association between dietary or serum copper and hs-CRP. It might be hypothesized that the copper-induced oxidative stress might determine an inflammatory response and that inflammation represents the result of copper damage instead of the cause for the increase in copper concentrations.

The increased mortality from cardiovascular disease in subjects with higher serum copper reported by epidemiological studies (8,9,33) clearly emphasizes the duplicitous nature of copper. Indeed, copper seems to be associated with a favorable metabolic pattern and there is now some interest in its potential insulin-mimetic effect (20). Although marginal copper deficiency may pose problems, copper supplementation might not be desirable due to its association with inflammation, markers of oxidative stress, and increased cardiovascular risk.

Limitations. Serum copper concentrations are perhaps not the best index of total body copper (3); however, the measurement of erythrocyte SOD activity is not a suitable biochemical marker for higher ranges of copper intake (29) and a cut-off has not yet been established in cases of severe copper deficiency in humans. Indeed, a reliable index of marginal copper status has not been identified.

We did not find an interaction between copper and zinc intake, but, in a previous study, zinc was shown to interact with copper only when supplemented at higher dosages (>30 mg/d) (34). A major limitation of cross-sectional studies is that even strong associations do not ensure causal relationships.

Simultaneous adjustments for correlated nutrients might be problematic, because both the regression coefficients and their standard errors tend to be unstable. However, no modifications of effects were found in our analyses after adjusting for dietary intakes of different nutrients (Table 3).

Only one measurement for each of the laboratory variables was taken and the validity and reliability of copper intake assessment by the SFFQ are unknown. Measurements errors, however, could have reduced the associations.

The possibility of uncontrolled or unknown confounders cannot be ruled out, even though we adjusted the data for various potential confounders.

Finally, the highly selected sample attenuates the possibility of bias and heterogeneity but reduces the ability to generalize our results.

Prospective long-term studies are required to assess links between copper and the incidence of diabetes, hypercholesterolemia, and cardiovascular diseases. At present, defining copper requirements may be problematic because of the adverse pro-oxidant effects of high copper intake and serum concentrations and, on other hand, the adverse metabolic pattern linked to marginal copper deficiency.

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