Nutrition, Metabolism & Cardiovascular Diseases (2023) **33**, 2199–2208

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

# Association between serum copper levels and risk of cardiovascular disease: A nested case-control study in the PREDIMED trial

Carlos Muñoz-Bravo [a,b,*], Pablo Olmedo [c], Fernando Gil [c], Miguel Ruiz-Canela [d,e], Miguel A. Martínez-González [d,e,f], María Ángeles Martínez [e,g], Nancy Babio [e,g,h], Montserrat Fitó [e,i], Jose L. del Val [j,k], Dolores Corella [e,l], José V. Sorlí [e,l], Emilio Ros [e,m], Miquel Fiol [e,n], Ramón Estruch [e,o], José Manuel Santos-Lozano [e,p], Fernando Arós [e,q], Luis Serra-Majem [e,r], Xavier Pintó [e,s], Enrique Gómez-Gracia [a,b], Mario Gutiérrez-Bedmar [a,b,t]

[a] *Department of Public Health and Psychiatry, School of Medicine, University of Málaga, Málaga, Spain*
[b] *Biomedical Research Institute of Malaga (IBIMA), Spain*
[c] *Department of Legal Medicine, Toxicology, and Physical Anthropology, School of Medicine, University of Granada, Granada, Spain*
[d] *Department of Preventive Medicine and Public Health, School of Medicine, University of Navarra, Pamplona, Spain*
[e] *CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid, Spain*
[f] *Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA*
[g] *Universitat Rovira i Virgili, Reus, Spain*
[h] *Human Nutrition Unit, Institud d'Investigació Sanitaria Pere Virgili, Spain*
[i] *Cardiovascular Risk and Nutrition Research Group. Hospital Del Mar Medical Research Institute (IMIM), Barcelona, Spain*
[j] *Unitat d'Avaluació, Sistemes d'Informació i Qualitat (BASIQ), Gerència Territorial Atenció Primària Barcelona Ciutat, Institut Català de la Salut, Barcelona, Spain*
[k] *Fundació Institut Universitari per a la Recerca a l'Atenció Primària de Salut Jordi Gol i Gurina (IDIAPJGol), Barcelona, Spain*
[l] *Department of Preventive Medicine, Universidad de Valencia, Valencia, Spain*
[m] *Lipid Clinic, Endocrinology and Nutrition Service, Institut d'Investigations Biomèdiques August Pi I Sunyer (IDIBAPS), Hospital Clínic, Barcelona, Spain*
[n] *Health Research Institute of the Balearic Islands (IdISBa), Hospital Son Espases, Palma de Mallorca, Spain*
[o] *Department of Internal Medicine, IDIBAPS, Hospital Clínic, University of Barcelona, Barcelona, Spain*
[p] *Department of Family Medicine, Research Unit, Distrito Sanitario Atención Primaria Sevilla, Sevilla, Spain*
[q] *Department of Cardiology, Hospital Universitario de Álava, Vitoria, Spain*
[r] *Department of Clinical Sciences & Research Institute of Biomedical and Health Sciences, Universidad de Las Palmas de Gran Canaria, Las Palmas, Spain*
[s] *Lipid and Vascular Risk Unit, Internal Medicine Service, Hospital Universitario de Bellvitge, Hospitalet de Llobregat, Spain*
[t] *CIBERCV Cardiovascular Diseases, Instituto de Salud Carlos III (ISCIII), Madrid, Spain*

*Received 18 May 2023; received in revised form 4 July 2023; accepted 6 July 2023*
*Handling Editor: A. Siani*
*Available online 17 July 2023*

**KEYWORDS**
Serum copper;
Trace elements;
Cardiovascular diseases;
Infarction;
Stroke;
PREDIMED

**Abstract** *Background and aim:* Certain trace elements have been associated with increased cardiovascular risk. The aim of this study was to evaluate the association between serum copper (S−Cu) levels and the risk of a first event of cardiovascular disease (CVD) in a population of older adults with high cardiovascular risk.
*Methods and results:* We conducted a case-control study nested within the PREDIMED trial. During a median follow-up of 4.8 years, a total of 207 incident cases diagnosed with CVD were matched for sex, age, and intervention group with 436 controls. Personal interviews, reviews of medical records, and validated questionnaires were used to assess known CVD risk factors. Biological serum samples were collected annually. Inductively coupled plasma mass spectrometry analysis was used to determine S−Cu levels. Adjusted odds ratios were calculated using multivariate conditional logistic regression models. All participants had S−Cu levels within the

*Abbreviations:* CVD, cardiovascular disease; PREDIMED, PREvención con DIeta MEDiterránea; S−Cu, serum copper; CI, confidence interval; aOR, adjusted odds ratio; BMI, body mass index.

* Corresponding author. Department of Public Health and Psychiatry, School of Medicine, University of Málaga, Boulevard Louis Pasteur 32, Málaga, 29071, Spain.
*E-mail address:* carlosmb@uma.es (C. Muñoz-Bravo).

https://doi.org/10.1016/j.numecd.2023.07.008

reference values, 750 μg/L to 1450 μg/L. Among men, but not among women, the mean S−Cu concentration was higher in cases 1014.1 μg/L than in controls 959.3 μg/L; (p = 0.004). In men, the multivariable-adjusted odds ratio for CVD was 2.36 (95% CI 1.07−5.20 for the comparison of the highest vs. the lowest quartile; p for trend = 0.02), in women, it was 0.43 (95% CI 0.11 −1.70; p for trend = 0.165).

*Conclusion:* In older Spanish men with high cardiovascular risk, a significant association was observed between high S−Cu levels, but still within the reference values, and an increased risk of a first event of CVD. Our findings suggest a sex difference in CVD risk and S−Cu levels. To confirm this relationship and to analyze the differences observed between men and women, further studies are needed.



## 1. Introduction

In people over 50 years of age, ischemic heart disease and stroke are the first and second leading causes of death worldwide, respectively [1]. Furthermore, during the last 20−30 years, the burden of disease attributed to cardiovascular pathology has increased worldwide, and this increase has been more pronounced in low-income countries [2]. Given this context, it is appropriate to identify new modifiable risk factors that help improve the effectiveness of primary prevention measures for cardiovascular disease (CVD). Scientific interest in studying the effect of different minerals, metals, and trace elements on CVD can be seen in various recently published studies [3−5].

Although the body's copper requirements are lower than other minerals, metals, and trace elements, it is still essential for many biological functions [6]. However, a disorder of copper metabolism can contribute to the development of different pathologies, such as CVD, cancer, and neurodegenerative disease [7].

A few previous studies [8−11] have analyzed the association between serum copper (S−Cu) levels and CVD, but the volume of scientific evidence in this regard is still limited and controversial. Thus, and to our knowledge, the isolated association of S−Cu levels with CVD has not been investigated in older adults with a high baseline risk of developing a cardiovascular event.

This article aimed to conduct a prospective case-control study nested within the PREDIMED trial [12,13] to examine the short-term (<2 years) association of S−Cu concentration within the reference values [14] with CVD risk (acute myocardial infarction, cerebrovascular accident or cardiovascular death) in a population of Spanish adults aged 55−80 years with a high risk of CVD.

## 2. Methods

### 2.1. Study design

A nested, matched case-control study was performed within the PREDIMED trial [12]. The PREDIMED trial is a multicenter, single-blind, controlled trial of primary prevention of CVD carried out in a population recruited in primary care health centers, with the aim of evaluating the effects of the Mediterranean diet on the incidence of CVD. The design and methods of the PREDIMED trial have been described in detail previously [12,15]. Briefly, 7447 participants at high cardiovascular risk were recruited who had no history of CVD at baseline. They were considered eligible if they met at least one of the following two criteria: a) type 2 diabetes mellitus or b) 3 or more of the following cardiovascular risk factors: current smoker, hypertension (blood pressure >140/90 mmHg or treatment with antihypertensives), high low-density lipoprotein (LDL) cholesterol level (>160 mg/dL or lipid-lowering treatment), low high-density lipoprotein (HDL) cholesterol level (<50 mg/dL in women and <40 mg/dL in men), body mass index (BMI) of ≥25 kg/m$^2$, or a family history of premature CVD. The exclusion criteria were: having a history of CVD, a serious chronic disease, low probability of making dietary changes according to the Prochaska and Diclemente stages of change model, among others. From October 2003 to June 2009, the participants were randomly assigned to one of the following groups: 1) Mediterranean diet supplemented with extra virgin olive oil; 2) Mediterranean diet supplemented with nuts; and 3) Control diet based on dietary advice to reduce fat intake. The PREDIMED trial was conducted according to the ethical standards guidelines of the Helsinki Declaration by the Institutional Review Boards of all participating centers. The institutional review board of the Hospital Clínic (Barcelona, Spain), which is accredited by the Department of Health and Human Services and regulated by the Federal wide Assurance for the Protection of Human Subjects of International (Non-US) Institutions (number 00000738), approved the study protocol on July 16, 2002. All participants provided written informed consent to authorize the use of biological samples for biochemical measurements. The trial was registered with the number ISRCTN35739639.

### 2.2. Assessment of cases and selection of controls

The primary clinical event evaluated as the trial's primary outcome was a composite of CVD events, defined as acute

myocardial infarction, stroke, or cardiovascular death. Four sources of information were used to identify the final events: 1) repeated contact with participants by phone and in person; 2) contact with the responsible family physician; 3) annual review of medical records; and 4) consultation of the National Death Index. Only final events confirmed by the adjudication committee, and that occurred between October 2003 (start of the PREDIMED study) and December 2010, were included in the analyses.

During follow-up, a total of 288 incident cases of CVD were identified. Sixty-two cases did not have serum samples available and were excluded from the analysis. For each case included, controls were selected by incidence density sampling and matched for age at blood collection (±2 years), sex, and intervention group. Participants with inadequate serum samples for mass spectrometry analysis (insufficient sample volume or poor sample quality) were excluded: 19 cases and 16 controls. The cases not included in the analysis did not differ in lifestyle or cardiovascular risk factors from the included CVD cases (Supplementary Table 1). The final sample size for the present analysis was 207 cases and 436 matched controls as follows: 188 case-control pairs at a ratio of 1:2, 17 pairs at a ratio of 1:1, and 2 pairs at a ratio of 1:3.

### 2.3. Blood sample collection and S–Cu measurement

Blood samples were collected from all participants after an overnight fast (>8 h) at baseline and at years 1, 3, 5, and 6 of follow-up (or at the final visit). Serum samples were processed and coded at each recruitment center, and aliquots were stored at −80 °C. Personnel responsible for sample processing and storage were blinded to each participant's intervention group. To assess short-term risk, S–Cu was measured by choosing the serum sample closest (previous) to the date of the event for each case. The median time between the serum sample collection and the appearance of a subsequent CVD event was 0.94 years (interquartile range: 0.38–1.96). Among controls, serum samples were chosen with a follow-up time similar to that of the corresponding case (risk set sampling).

For S–Cu analysis, a calibration curve was prepared using a 1000 μg/mL Cu standard solution (High-Purity Standards, Charleston, SC, USA) in a 2% (w/v) solution, 1-butanol (Merck, Darmstadt, Germany), 0.05% (w/v), EDTA (Aldrich, St. Louis, MO, USA) 0.05% (w/v) Triton X-100 (Merck, Darmstadt, Germany), and 1% (w/v) NH4OH (Merck, Darmstadt, Germany) in ultrapure water (Milli-Q, Merck, Darmstadt, Germany). Serum samples were diluted 1:10 in the solution mentioned above. The S–Cu analysis was performed using an Agilent 8900 (Agilent Technologies, Santa Clara, CA, USA) triple-quadrupole inductively coupled plasma mass spectrometer. The instrument was set, and performance parameters were checked prior to analysis. To ensure the quality of the results, 40 mg/L germanium (ISC Science, Oviedo, Spain) was added to the samples as an internal standard. In addition, a suitable certified reference material [Seronorm (Sero, Billingstad, Norway) Trace Elements Serum L2 (reference 203,105)] was re-analyzed along with a blank and intermediate calibration standard every 12 samples. The National Institute of Standards and Technology NIST (USA) Trace Elements in Natural Water Standard Reference Material SRM 1640a was used as the certified reference material and was analyzed at the beginning and end of each sequence. Additionally, one out of 12 samples was retested at the end of each session. Repeat measurement of a certified standard serum sample yields an intra-assay coefficient of variation of 4.67% and an inter-assay coefficient of variation of 3.67%. The detection limit for copper was 0.18 μ/L, and there were no concentrations below the detection limit.

Participants' glucose, triglyceride, total cholesterol, LDL cholesterol, and HDL cholesterol levels were determined locally using fasting plasma samples at baseline. LDL cholesterol levels were calculated using the Friedewald formula whenever triglyceride levels were <300 mg/dL.

### 2.4. Evaluation of covariables

Trained dieticians completed the following using personal interviews: 1) A 47-item questionnaire on variables related to lifestyles, medical history, and medication use. Medications commonly used by the participants were grouped into 8 categories: angiotensin-converting enzyme inhibitors; diuretics; statins; insulin; acetylsalicylic acid; calcium channel blockers; angiotensin II receptor antagonists; and beta-blockers; 2) A validated 14-item questionnaire designed to assess adherence to a Mediterranean diet pattern [16]; 3) A semi-quantitative questionnaire of food consumption frequencies, previously validated, with 137 items [17]. For the analysis, the consumption of the following food groups was considered: meat, fish, vegetables, legumes, fruits, cereals, dairy products, and nuts. The total intake of energy and nutrients was calculated using the information collected in the Spanish food composition tables [18]; 4) The validated Spanish version of the Minnesota leisure-time physical activity questionnaire [19,20]. In addition, trained personnel performed anthropometric measurements and measured blood pressure according to the study protocol. The questionnaires and the anthropometric measurements were conducted at the beginning of the study and annually during the follow-up visits.

### 2.5. Statistical analysis

Case-control characteristics were described as mean and standard deviation for continuous variables and absolute and relative frequencies for categorical variables. Group comparisons were made using the Student's t-test or Chi-square test, as appropriate. S–Cu levels (μg/L) were categorized into sex-specific quartiles with cutoff points based on the distribution in the control group [21]. Adjusted levels of covariates across S–Cu quartiles in controls were estimated by analysis of variance. Polynomial contrasts (linear trend) were used to evaluate the association of these adjusted levels with the S–Cu quartiles.

To estimate the association between S–Cu concentrations and CVD risk, we used three conditional logistic regression models (conditional on matching) with successive degrees of fit: a) Adjusted only for matching factors; b) Adjustment for cardiovascular risk factors and potential confounders based on clinical relevance and prior causal knowledge; and c) absence of multicollinearity. Variables associated with S–Cu levels at a level of statistical significance $p < 0.25$ were also included in the maximum fit model [22]. Sex-specific S–Cu quartiles were included in the models as a categorical variable, and crude odds ratio and adjusted Odds Ratio (aOR) and their 95% confidence intervals (CI) were estimated for the three upper quartiles, always using the lowest quartile as the reference category. Trend tests were performed by assigning each participant the median value of the S–Cu quartile and treating it as a continuous variable.

The possible interaction between S–Cu levels with sex and age was investigated. The statistical significance of the interactions was evaluated using the likelihood ratio test based on the models with and without the interaction terms.

We also used conditional logistic regression adjusted for multiple variables to explore the relationship between dietary copper intake and CVD. We further explored the possible non-linear association between S–Cu levels and CVD risk using the restricted cubic spline analysis.

All hypothesis tests were bilateral. A p-value $<0.05$ was considered statistically significant. All statistical analyses were conducted using Stata 17.0 (Stata Corp).

## 3. Results

### 3.1. Participant characteristics at the time of blood sample collection

Fig. 1 shows the flowchart of the participants included in the final analyses.

The characteristics of the study population at the time of sample collection for the determination of S–Cu are shown in Table 1. The cases as a whole presented mean levels of S–Cu higher than the controls (1052.4 μg/L vs. 1016.5 μg/L; $p = 0.024$). However, when differentiating between men and women, the difference in S–Cu levels between cases and controls was observed only in men, with a mean S–Cu concentration of 1014.12 μg/L in cases, while in controls, it was 959.26 μg/L ($p = 0.004$).

The frequency of cardiovascular risk factors (smoking, hypercholesterolemia, hypertension, diabetes, mean blood glucose, and triglyceride levels) was higher in the cases than in the controls, as expected. The same occurred with the consumption of acetylsalicylic acid as antiplatelet treatment (33.8% vs. 22.0%; $p = 0.001$). However, the use of statins was significantly less frequent in the cases than in the controls (25.1% vs. 38.5%; $p = 0.001$).

Regarding dietary characteristics related to copper intake (intake of viscera and shellfish), no differences were observed between cases and controls in the crude analysis.

### 3.2. Lifestyle and dietary factors associated with S–Cu in controls

Table 2 summarizes the characteristics of the control group based on S–Cu levels adjusted for age, sex, and the recruitment center of the controls. A statistically significant inverse association was observed between fruit consumption and copper levels (difference Q4 vs. Q1 = −48.64 95% CI (−95.9 to −1.4); p for linear trend = 0.03). A similar inverse association was also observed for fish (difference Q4 vs. Q1 = −11.77 95% CI (−23.3 to −0.2); p for linear trend = 0.02). No statistically significant association was found for the rest of the dietetic and lifestyle factors analyzed.

### 3.3. S–Cu and CVD risk

The association between S–Cu levels and CVD risk is shown in Table 3. When not adjusting for potential confounders, a higher CVD risk was found at higher S–Cu levels. However, sex seemed to act as an effect modifier, but with only marginal statistical significance for the end product (interaction $p = 0.086$).

Although no association was observed in women, in men, a direct association was found between S–Cu levels and CVD risk (aOR Q4 vs. Q1 = 2.36, 95% CI: 1.07–5.20; p for trend = 0.02). The interaction between copper levels and the categorized Mediterranean diet adherence score was investigated, as well as with the categorized BMI, with no differences of interest being found (data not shown).

The dose-response relationship between S–Cu levels and CVD risk was evaluated in the restricted cubic spline analysis based on the maximum fit model (Supplementary Fig. 1). Non-linear association was observed for total sample (p for non-linearity = 0.003). Estimated curve showed a non-significant positive linear relationship up to concentrations of approximately 1100 μg/L, after which the risk remained constant. Linear associations were observed for men and women samples (p for non-linearity = 0.235 and 0.349, respectively).

## 4. Discussion

An adverse relationship between S–Cu levels and CVD risk was observed in men aged 55–80 years in this case-control study nested the PREDIMED trial. No adverse relationship was found for women. S–Cu levels were within the reference values in all participants. They depend mainly on dietetic factors, such as organ meats and shellfish consumption. Hence future studies analyzing the role of consumption of these foods and cardiovascular risk in other populations are warrant.

It is important to note that the potential interaction by the sex variable was only marginally significant. However, we cannot rule out that our findings result from a false positive due to the low number of events in women. Furthermore, plasma copper concentrations are usually higher in women than in men, which may make it difficult to identify associations in women. Age, type of diet,

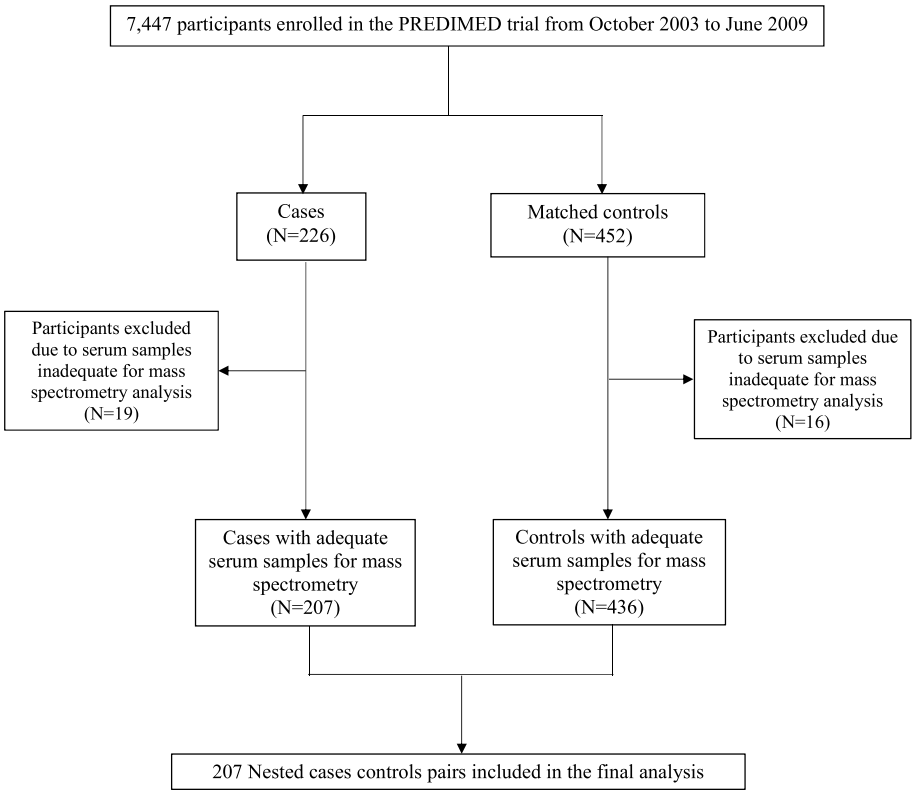


**Figure 1** Flowchart of the study participants: a nested case-control design based on the PREDIMED, "PREvención con DIeta MEDiterránea".

amount of dietary copper, the use of oral contraceptives, and the hormonal profile may result in a different efficiency in the rate of absorption and excretion of copper by men and women [23] [–] [25]. Nonetheless, Ford ES et al., the only study we were able to locate that analyzes the role of S–Cu for men and women and the risk of cardiovascular mortality, found results similar to ours, describing a higher risk only in men but no association in women, as we observed. However, there is no solid pathophysiological basis supporting a real differential relationship of S–Cu levels according to sex or to rule it out. Hence, with only 2 studies supporting this effect modification by sex, there is a need for future replication of this interaction in additional studies. In our data, the lack of statistical power in women may explain the lack of significant association described. However, the specific reasons that may explain the observed differences in CVD risk between men and women are still unknown. As with other metals such as lead, cadmium, arsenic or mercury, possible differences in the kinetics and mode of action may be related to the observed differences [26]. Furthermore, changes related to sex hormones [27,28], as well as factors related to renal function [29] may be involved in the observed sex-differences.

The relationship between plasma copper levels and CVD has been analyzed previously, although the results are not always consistent [30] [–] [32]. Two recent meta-analyses suggest a direct association between elevated S–Cu levels and CVD [33,34]. Two other systematic reviews and meta-analyses found that S–Cu levels are higher in subjects with acute myocardial infarction [31] and ischemic stroke than in the healthy population [32], although there are studies that find lower levels in patients with hemorrhagic stroke [30]. However, the small sample size, the measurement of S–Cu levels at the time of diagnosis, the non-assessment of publication bias or the impossibility of ruling it out in the meta-analyses carried out, the non-consideration of CVD as a whole or the behavior of the association differently depending on the type of population (no association in the Caucasian population for acute myocardial infarction), justify the need for its analysis in the European population based on the PREDIMED study.

Most studies that analyze the association between S–Cu and CVD, the relative risks ranged from 1.15 to a maximum of 4.0, although, in some cases, statistical significance is not reached. The comparison of risk between studies is not easy for different reasons: 1) Type of design, based on a cohort design [8,9,35,36] or nested case-control study [10,11,37]; 2) type of population, while some studies only include males [9,35–37] others include both sexes [3,11,38–40]; 3) type of result variable, cerebrovascular disease [10,11,41], myocardial infarction [35] or CVD as a whole [9,36,37,40]; 4) Measurement of exposure, there is no established cutoff point for S–Cu levels to determine whether levels are optimal or not. For this reason, studies use internal comparison criteria based on the characteristics of the population, quartiles [9,11,36] or terciles [8,10,35,37].

**Table 1** Characteristics of cases and matched controls at sample collection time. The PREDIMED trial.

| Characteristic | Case participants | Control participants | p value |
|---|---|---|---|
| N | 207 | 436 | |
| Age (years) | 70.9 (6.8) | 71.3 (6.6) | Matching Factor |
| Sex (% women) | 37.2 | 38.5 | Matching Factor |
| PREDIMED trial arm (%) | | | |
| Mediterranean diet + EVOO | 32.4 | 31.0 | Matching Factor |
| Mediterranean diet + nuts | 27.1 | 27.5 | Matching Factor |
| Serum copper (mg/L) | 1052.4 (187.5) | 1016.5 (188.8) | **0.024** |
| Men (n = 398) | 1014.1 (182.8) | 959.3 (170.3) | **0.004** |
| Women (n = 245) | 1117.1 (178.5) | 1107.8 (181.1) | 0.709 |
| Smoking status (%) | | | |
| Current | 20.3 | 11.9 | **0.005** |
| Former | 37.2 | 34.2 | |
| Non-smokers | 42.5 | 53.9 | |
| Hypercholesterolemia (%) | 44.0 | 35.3 | **0.035** |
| Hypertension (%) | 61.8 | 50.9 | **0.009** |
| Type 2 diabetes (%) | 62.8 | 53.2 | **0.022** |
| Family history of CHD (%) | 19.8 | 20.9 | 0.755 |
| Body mass index (kg/m$^2$) | 29.5 (3.6) | 29.2 (3.4) | 0.305 |
| Physical activity (METs-min/day) | 239.9 (238.4) | 275.6 (262.3) | 0.097 |
| Alcohol (g/day) | 8.8 (15.1) | 10.8 (14.6) | 0.117 |
| Biochemical parameters | | | |
| Glucose[a] (mg/dL) | 137.7 (52.0) | 124.2 (36.9) | **0.003** |
| Triglycerides[a] (mg/dL) | 147.6 (82.6) | 130.4 (60.7) | **0.017** |
| Total cholesterol[a] (mg/dL) | 202.4 (33.4) | 205.4 (38.9) | 0.453 |
| HDL cholesterol[a] (mg/dL) | 49.0 (10.1) | 50.7 (9.8) | 0.107 |
| Mediterranean diet adherence (0–14) | 9.44 (2.1) | 9.8 (2.0) | 0.058 |
| Dietary intake | | | |
| Total energy intake (kcal/day) | 2301.3 (646.2) | 2283.3 (560.6) | 0.717 |
| Total Fat (%E) | 40.1 (6.8) | 40.0 (6.6) | 0.951 |
| Monounsaturated Fat (%E) | 20.3 (4.5) | 20.5 (4.4) | 0.611 |
| Polyunsaturated Fat (%E) | 6.5 (2.1) | 6.5 (2.0) | 0.889 |
| Saturated Fat (%E) | 9.8 (2.3) | 9.5 (2.1) | 0.096 |
| Carbohydrates (%E) | 40.9 (7.3) | 40.7 (6.9) | 0.680 |
| Cholesterol (mg/day) | 373.4 (142.7) | 358.1 (129.4) | 0.176 |
| Fiber (g/day) | 25.3 (9.3) | 25.4 (7.7) | 0.843 |
| Total meat (g/day) | 132.3 (62.8) | 124.8 (51.7) | 0.111 |
| Total fish (g/day) | 100.9 (50.9) | 104.0 (46.4) | 0.441 |
| Total legumes (g/day) | 23.6 (15.5) | 22.5 (14.4) | 0.363 |
| Total vegetables (g/day) | 314.7 (133.5) | 319.2 (140.7) | 0.702 |
| Total fruit (g/day) | 383.1 (207.7) | 392.0 (181.1) | 0.577 |
| Total cereals (g/day) | 225.7 (104.3) | 228.7 (111.4) | 0.750 |
| Total dairy (g/day) | 379.8 (214.6) | 358.0 (216.0) | 0.231 |
| Total nuts (g/day) | 5.5 (8.3) | 6.7 (10.0) | 0.127 |
| Liver (g/day) | 1.6 (4.4) | 1.3 (3.3) | 0.289 |
| Bivalves (g/day) | 2.7 (4.4) | 2.9 (4.2) | 0.522 |
| Educational level (%) | | | |
| Primary or less | 77.8 | 78.09 | 0.941 |
| Secondary | 14.0 | 13.5 | |
| Tertiary | 8.2 | 7.6 | |
| Medication use (%) | | | |
| ACE inhibitors | 34.8 | 30.7 | 0.304 |
| Diuretics | 20.3 | 22.3 | 0.573 |
| Statins | 25.1 | 38.5 | **0.001** |
| Insulin | 7.7 | 5.5 | 0.275 |
| Aspirin-antiplatelet drugs | 33.8 | 22.0 | **0.001** |
| Calcium channel blockers | 19.3 | 15.4 | 0.208 |
| Angiotensin II receptor antagonists | 17.4 | 19.5 | 0.524 |
| Beta-blockers | 13.5 | 9.9 | 0.166 |

EVOO: extra-virgin olive oil, CHD: coronary heart disease; METs: metabolic equivalents; %E: percentage of total energy intake; PREDIMED: PREvención con DIeta MEDiterránea.
Data given as mean (standard deviation) or %.
Statistically significant results are shown in bold ($p < 0.05$).
[a] Basal measurement.

**Table 2** Adjusted[a] characteristics of 463 controls by sex-specific quartiles of serum copper at sample collection time.

| Variables | Quartiles[b] of serum copper | | | | p for linear trend | Difference Q4 vs Q1 (95% IC) |
|---|---|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 | | |
| No. of participants | 109 | 109 | 109 | 109 | | |
| Median serum copper level (μg/L) | | | | | | |
| Men (n = 268) | 776.3 | 900.8 | 984.2 | 1155.4 | NA | NA |
| Women (n = 168) | 902.8 | 1059.0 | 1162.1 | 1301.8 | NA | NA |
| Age[c] (years) | 70.4 | 71.7 | 71.6 | 71.60 | 0.22 | 1.2 (−0.6–2.9) |
| PREDIMED trial arm (%) | | | | | | |
| Mediterranean diet + EVOO | 22.0 | 33.0 | 38.5 | 30.4 | 0.12 | 8.4 (−4.0–20.7) |
| Mediterranean diet + nuts | 28.5 | 26.6 | 21.1 | 33.9 | 0.58 | 5.4 (−6.6–17.3) |
| Smoking status (%) | | | | | | |
| Current | 8.9 | 11.1 | 12.0 | 15.7 | 0.12 | 6.8 (−1.6–15.2) |
| Former | 36.4 | 28.7 | 36.8 | 34.8 | 0.84 | −1.6 (−12.5–9.3) |
| Never | 54.7 | 60.3 | 51.2 | 49.5 | 0.13 | −5.2 (−15.3–4.9) |
| Hypercholesterolemia (%) | 33.4 | 40.2 | 44.0 | 33.7 | 0.79 | 0.3 (−11.9–12.6) |
| Hypertension (%) | 45.2 | 55.8 | 46.8 | 56.0 | 0.28 | 10.8 (−2.5–24.0) |
| Type 2 diabetes (%) | 57.3 | 58.5 | 47.6 | 49.5 | 0.11 | −7.8 (−21.0–5.3) |
| Family history of CHD (%) | 22.9 | 22.2 | 21.3 | 17.2 | 0.30 | −5.7 (−16.4–5.0) |
| Body mass index (kg/m$^2$) | 29.4 | 29.5 | 29.2 | 29.0 | 0.31 | −0.4 (−1.3–0.5) |
| Physical activity (METs-min/day) | 261.9 | 283.0 | 292.6 | 265.1 | 0.86 | 3.2 (−65.4–72.0) |
| Alcohol (g/day) | 10.9 | 10.8 | 10.5 | 11.0 | 0.98 | 0.1 (−3.5–3.7) |
| Biochemical parameters | | | | | | |
| Glucose[d] (mg/dL) | 126.3 | 122.2 | 121.6 | 126.5 | 0.99 | 0.2 (−12.0–12.3) |
| Triglycerides[d] (mg/dL) | 125.7 | 136.0 | 133.1 | 127.1 | 0.97 | 1.4 (−18.8–21.5) |
| Total cholesterol[d] (mg/dL) | 204.1 | 208.1 | 201.7 | 207.5 | 0.86 | 3.4 (−9.5–16.3) |
| HDL cholesterol[d] (mg/dL) | 50.2 | 50.6 | 50.0 | 52.0 | 0.32 | 1.8 (−1.3–5.0) |
| Mediterranean diet adherence (0–14) | 9.8 | 9.6 | 9.7 | 10.0 | 0.46 | 0.2 (−0.4–0.7) |
| Dietary intake | | | | | | |
| Total energy intake (kcal/day) | 2271.4 | 2206.1 | 2344.4 | 2311.2 | 0.27 | 39.8 (−106.1–185.7) |
| Total Fat (%E) | 40.0 | 40.2 | 40.1 | 39.9 | 0.86 | −0.1 (−1.7–1.6) |
| Monounsaturated Fat (%E) | 20.3 | 20.7 | 20.5 | 20.5 | 0.88 | 0.2 (−0.9–1.3) |
| Polyunsaturated Fat (%E) | 6.5 | 6.5 | 6.6 | 6.3 | 0.61 | −0.2 (−0.7–0.3) |
| Saturated Fat (%E) | 9.6 | 9.5 | 9.4 | 9.5 | 0.58 | −0.1 (−0.7–0.4) |
| Carbohydrates (%E) | 40.4 | 40.4 | 41.1 | 40.8 | 0.55 | 0.4 (−1.4–2.1) |
| Cholesterol (mg/day) | 357.5 | 349.9 | 362.3 | 362.6 | 0.62 | 5.1 (−29.3–39.5) |
| Fiber (g/day) | 25.6 | 24.8 | 26.12 | 25.0 | 0.93 | −0.6 (−2.6–1.5) |
| Total Meat[e] (g/day) | 125.7 | 118.0 | 125.0 | 130.6 | 0.32 | 4.9 (−8.6–18.5) |
| Total fish[e] (g/day) | 109.9 | 109.0 | 99.2 | 98.1 | 0.02 | −11.8 (−23.3 – (−0.2)) |
| Total legumes[e] (g/day) | 22.4 | 21.1 | 22.3 | 24.3 | 0.26 | 1.9 (−1.9–5.8) |
| Total vegetables[e] (g/day) | 303.6 | 316.2 | 344.4 | 312.7 | 0.36 | 9.1 (−28.2–46.5) |
| Total fruit[e] (g/day) | 412.6 | 405.6 | 386.0 | 364.0 | 0.06 | −48.6 (−95.9 – (−1.4)) |
| Total cereals[e] (g/day) | 213.7 | 225.8 | 248.0 | 227.1 | 0.18 | 13.4 (−15.6–42.3) |
| Total dairy[e] (g/day) | 362.1 | 334.2 | 359.9 | 375.8 | 0.47 | 13.7 (−43.7–71.1) |
| Total nuts[e] (g/day) | 7.5 | 6.3 | 6.6 | 6.5 | 0.55 | −1.0 (−3.6–1.7) |
| Liver (g/day) | 1.1 | 1.0 | 1.3 | 1.7 | 0.12 | 0.6 (−0.3–1.5) |
| Bivalves (g/day) | 2.8 | 3.8 | 2.4 | 2.8 | 0.49 | 0.0 (−1.1–1.2) |
| Educational level (%) | | | | | | |
| Primary or less | 78.7 | 79.4 | 76.8 | 80.7 | 0.84 | 2.0 (−8.7–12.8) |
| Secondary | 14.0 | 13.2 | 15.8 | 11.1 | 0.67 | −2.9 (−12.0–6.1) |
| Tertiary | 7.3 | 7.4 | 7.4 | 8.2 | 0.81 | 0.9 (−6.2–8.0) |
| Medication use (%) | | | | | | |
| ACE inhibitors | 29.7 | 26.2 | 34.6 | 32.5 | 0.394 | 2.8 (−9.5–15.1) |
| Diuretics | 21.2 | 19.4 | 23.9 | 24.5 | 0.421 | 3.3 (−7.8–14.3) |
| Statins | 35.8 | 44.8 | 33.9 | 39.7 | 0.964 | 3.9 (−9.0–16.9) |
| Insulin | 2.8 | 6.3 | 4.5 | 8.4 | 0.132 | 5.6 (−0.6–11.6) |
| Aspirin-antiplatelet drugs | 22.9 | 23.4 | 17.1 | 24.7 | 0.960 | 1.8 (−9.2–12.8) |
| Calcium channel blockers | 13.1 | 15.7 | 16.5 | 16.2 | 0.515 | 3.1 (−6.6–12.8) |
| Angiotensin II receptor antagonists | 15.6 | 28.3 | 18.3 | 15.8 | 0.573 | 0.2 (−10.4–10.7) |
| Beta-blockers | 10.1 | 10.2 | 6.5 | 12.8 | 0.731 | 2.7 (−5.3–10.7) |

EVOO: extra-virgin olive oil, CHD: coronary heart disease; METs: metabolic equivalents; %E: percentage of total energy intake; ACE: Angiotensin converting enzyme inhibitors; 95% CI: 95% confidence interval.
Data given as mean or %.
Statistically significant results are shown in bold ($p < 0.05$).
[a] Adjusted for age, sex and center.
[b] Sex-specific quartiles of serum copper based on distribution among controls. Cut-off values of serum copper were: 851.66, 933.98 and 1061.38 μg/L in men and 992.94, 1127.37 and 1219.23 μg/L in women.
[c] Adjusted for age and center.
[d] Basal measurement.
[e] Adjusted for age, sex, center, body mass index and total energy intake.

**Table 3** Adjusted odds ratios for cardiovascular disease by sex-specific quartiles of serum copper. PREDIMED trial.

| Quartiles[a] of serum copper | | | | | | |
|---|---|---|---|---|---|---|
| | Number of cases/controls | Q1 | Q2 | Q3 | Q4 | p for trend |
| Total population | | | | | | |
| Cases/matched controls | 207/436 | 43/109 | 43/109 | 55/109 | 66/109 | |
| Matched OR[b] (95% CI) | | 1 (Ref.) | 0.98 (0.59–1.62) | 1.19 (0.73–1.97) | 1.56 (0.96–2.55) | **0.034** |
| Matched OR[c] (95% CI) | | 1 (Ref.) | 0.94 (0.54–1.64) | 1.19 (0.69–2.07) | 1.37 (0.80–2.34) | 0.157 |
| Matched OR[d] (95% CI) | | 1 (Ref.) | 1.04 (0.59–1.84) | 1.14 (0.65–2.03) | 1.28 (0.73–2.25) | 0.336 |
| Men | | | | | | |
| Cases/matched controls | 130/268 | 23/67 | 23/67 | 41/67 | 43/67 | |
| Matched OR[e] (95% CI) | | 1 (Ref.) | 1.01 (0.51–1.97) | 1.78 (0.94–3.40) | **1.97 (1.03–3.77)** | **0.013** |
| Matched OR[c] (95% CI) | | 1 (Ref.) | 1.03 (0.50–2.14) | 1.98 (0.98–4.01) | **2.18 (1.06–4.48)** | **0.013** |
| Matched OR[d] (95% CI) | | 1 (Ref.) | 1.30 (0.59–2.89) | **2.19 (1.01–4.72)** | **2.36 (1.07–5.20)** | **0.020** |
| Women | | | | | | |
| Cases/matched controls | 77/168 | 20/42 | 20/42 | 14/42 | 23/42 | |
| Matched OR[e] (95% CI) | | 1 (Ref.) | 0.94 (0.43–2.05) | 0.60 (0.26–1.40) | 1.10 (0.51–2.37) | 0.957 |
| Matched OR[c] (95% CI) | | 1 (Ref.) | 0.94 (0.31–2.82) | 0.41 (0.12–1.39) | 0.81 (0.27–2.42) | 0.513 |
| Matched OR[d] (95% CI) | | 1 (Ref.) | 0.64 (0.15–2.72) | 0.26 (0.06–1.16) | 0.43 (0.11–1.70) | 0.165 |

Statistically significant results are shown in bold ($p < 0.05$).
[a] Sex-specific quartiles of serum copper based on distribution among controls.
[b] Models are from conditional logistic regressions analyses with matching factors sex, age and intervention group.
[c] Adjusted for center (indicator variable), smoking (binary), hypertension, hypercholesterolemia, diabetes, family history of premature coronary heart disease, body mass index (Kg/m$^2$), physical activity (METs-min/day) and alcohol intake (g/day).
[d] Additionally adjusted for liver intake (g/day), total energy intake (Kcal/day), adherence to the Mediterranean diet (0–14 points) insulin (binary), total fish (g/day), total fruit (g/day), total cereals (g/day) and statins (binary).
[e] Model are from conditional logistic regression analyses with matching factors age and intervention group.

These differences make it challenging to determine the actual magnitude of the association, although the association between higher levels of S–Cu and CVD in men seems consistent. In our study, a positive trend is observed between S–Cu and CVD levels, with double the risk in men with a level higher than 1061.38 μg/L compared to the reference category with a level lower than 851.66 μg/L, aOR Q4 vs. Q1 = 2.37 (95% CI: 1.09–5.19; p for trend = 0.01). Different mechanisms have been described that may explain this relationship. Copper has the ability to produce reactive oxygen and nitrogen species through redox and Fenton reactions, which favors the lipid peroxidation process and contributes to the development of atherosclerotic processes [7]. Additionally, copper participates in the modulation of the inflammatory response [9,42]. Specifically, it amplifies the inflammatory response through the extracellular release of reactive species that activate cytokines, chemokines, and endothelial leukocyte adhesion molecules. Some studies have reported an association between S–Cu and inflammation markers such as high-sensitivity C-reactive protein and α-1 acid glycoprotein [42]. Likewise, it has been reported that the plasmatic copper-homocysteine interaction contributes to hydrogen peroxide favoring the development of peripheral arterial disease and ischemic heart disease [43,44]. Obesity should be added to these mechanisms, with which it shares common biochemical mechanisms involved in the underlying inflammatory processes in the development of CVD [42,45].

Several the limitations of our study should be acknowledged: 1) Our study included a population of Spanish adults at high risk of CVD. This may make it difficult to generalize the results to other populations; 2) The inflammation status could behave as a potential confounding factor not measured in our study; 3) The main source of copper intake is diet. The present work, integrated into the PREDIMED study, is based on a cohort subjected to dietary intervention, which could condition both copper levels as well as the incidence of CVD. To control its effect, the intervention group has been considered as a matching variable, minimizing this effect; 4) Although all comparisons were based on an a priori planned contrast, due to the number of comparisons made, the possibility of an increase in overall alpha risk due to multiple tests cannot be ruled out; 5) Regarding the determination of copper levels, it may be that S–Cu is not the measure that best reflects the levels of metabolically active copper in body tissues [23,28]; 6) The S–Cu measurement was limited to a single occasion, with the consequent effect that intra-individual variability itself may introduce.

Among the strengths, it should first be noted that the PREDIMED study itself, represents a large trial with more than 4 years of follow-up, a well-characterized population, a precise and blind evaluation of incident cases of CVD, and the monitoring of a large number of possible confounding variables [12]. In this regard, a novelty of our study is that, to our knowledge, this is the first study that analyzes the association between different food groups with S–Cu levels in the context of the analysis of the S–Cu association and CVD risk. This may help explain the relationship between certain types of food and CVD risk. On

the other hand, given the possible variation in copper levels throughout the day, blood samples were always drawn in the morning, after a fasting period of not less than 8 h and at approximately the same time (from 8:00 a.m. to 9:00 a.m.). In addition, parameter estimation and multivariable models were adjusted by study center to account for possible differences in sample handling. Finally, using incidence density sampling also minimizes the possibility of selection bias from the control group. The short period between the measurement of S–Cu and the occurrence of the event (less than 2 years) allows for a short-term assessment of CVD risk.

In summary, in this case-control study nested in the PREDIMED trial, a dose-response gradient is observed between high levels of S–Cu, although still within the reference values [14], and CVD risk in older adult men. Further studies are needed to confirm this relationship and analyze the differences observed between men and women to verify whether the suggestion of modification of the effect by sex is replicated in independent studies.

## Declaration of competing interest

Authors declare no conflict of interest in this article.

## Funding information

This research was funded by the official funding agency for biomedical research of the Spanish government, Instituto de Salud Carlos III (ISCIII), through grants provided to research networks specifically developed for the trial (RTIC G03/140; RTIC RD 06/0045 “PREDIMED”), and JR14/00008, and through Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBERobn), and by grants from Centro Nacional de Investigaciones Cardiovasculares (CNIC06/2007), the Fondo de Investigación Sanitaria–Fondo Europeo de Desarrollo Regional (Proyecto de Investigación (PI04-2239, PI05/2584, CP06/00100, PI07/0240, PI07/1138, PI07/0954, PI 07/0473, PI10/01407, PI10/02658, PI11/01647, P11/02505 and PI13/00462), the Ministerio de Ciencia e Innovación (Recursos y teconologia agroalimentarias (AGL)-2009-13906-C02 and AGL2010-22319-C03 and AGL2013-49083C3-1-R), the Ministerio de Economía y Competitividad-Fondos FEDER-Instituto de Salud Carlos III (UNGR15-CE-3380), the Fundación Mapfre 2010, the Consejería de Salud de la Junta de Andalucía (PI0105/2007), the Public Health Division of the Department of Health of the Autonomous Government of Catalonia, the Generalitat Valenciana (Generalitat Valenciana Ayuda Complementatia GVACOMP) 06109, GVACOMP2010-181, GVACOMP2011-151), Conselleria de Sanitat y AP; Atención Primaria (CS) 2010-AP-111, and CS2011-AP-042), Regional Government of Navarra (P27/2011), and Centre Català de la Nutrició de l’Institut d’Estudis Catalans. Hojiblanca and Patrimonio Communal Olivarero donated extra-virgin olive oil; the California Walnut Commission donated walnuts; Borges donated almonds; La Morella Nuts donated hazelnuts.

## Acknowledgments

The authors thank the participants for their enthusiastic collaboration, the PREDIMED personnel for excellent assistance, D. José Santiago Rodriguez from the University of Granada for his help in the sample analysis, and the personnel of all affiliated primary care centers.

The authors would like to thank the Universidad de Malaga/ Consorcio de Bibliotecas Universitarias de Andalucía (CBUA) for funding the Open Access fees for the publication.

The authors would like to thank the Aula de Dieta Mediterránea y Vida Saludable de la Real Academia de Medicina de Andalucía Oriental, Ceuta y Melilla for its financial support in the analytical determination of copper levels.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2023.07.008.

## References

[1] Abbafati C, Abbas KM, Abbasi-Kangevari M, Abd-Allah F, Abdelalim A, Abdollahi M, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 2020; 396:1204–22. https://doi.org/10.1016/S0140-6736(20)30925-9.

[2] World Health Organization. WHO - the top 10 causes of death. n.d. https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death. [Accessed 26 September 2022].

[3] Shi L, Yuan Y, Xiao Y, Long P, Li W, Yu Y, et al. Associations of plasma metal concentrations with the risks of all-cause and cardiovascular disease mortality in Chinese adults. Environ Int 2021;157:106808. https://doi.org/10.1016/j.envint.2021.106808.

[4] Gutierrez-Bedmar M, Olmedo P, Gil F, Ruiz-Canela M, Martínez-González MA, Salas-Salvadó J, et al. Low serum iron levels and risk of cardiovascular disease in high risk elderly population: nested case–control study in the PREvención con DIeta MEDiterránea (PREDIMED) trial. Clin Nutr 2021;40:496–504. https://doi.org/10.1016/j.clnu.2020.05.044.

[5] Yang L, Chen X, Cheng H, Zhang L. Dietary copper intake and risk of stroke in adults: a case-control study based on national health and nutrition examination survey 2013–2018. Nutrients 2022;14. https://doi.org/10.3390/nu14030409.

[6] Uauy R, Olivares M, Gonzalez M. Essentiality of copper in humans. Am J Clin Nutr 1998;67:952–9. https://doi.org/10.1093/ajcn/67.5.952S.

[7] Chen J, Jiang Y, Shi H, Peng Y, Fan X, Li C. The molecular mechanisms of copper metabolism and its roles in human diseases. Pflügers Archiv 2020;472:1415–29. https://doi.org/10.1007/s00424-020-02412-2.

[8] Marniemi J, Alanen E, Impivaara O, Seppänen R, Hakala P, Rajala T, et al. Dietary and serum vitamins and minerals as predictors of myocardial infarction and stroke in elderly subjects. Nutr Metabol Cardiovasc Dis 2005;15:188–97. https://doi.org/10.1016/j.numecd.2005.01.001.

[9] Leone N, Courbon D, Ducimetiere P, Zureik M. Zinc, copper, and magnesium and risks for all-cause, cancer, and cardiovascular mortality. Epidemiology 2006;17:308–14. https://doi.org/10.1097/01.ede.0000209454.41466.b7.

[10] Xiao Y, Yuan Y, Liu Y, Yu Y, Jia N, Zhou L, et al. Circulating multiple metals and incident stroke in Chinese adults: the dongfeng-Tongji cohort. Stroke 2019;50:1661–8. https://doi.org/10.1161/STROKEAHA.119.025060.

[11] Hu L, Bi C, Lin T, Liu L, Song Y, Wang P, et al. Association between plasma copper levels and first stroke: a community-based nested case–control study. Nutr Neurosci 2022;25:1524–33. https://doi.org/10.1080/1028415X.2021.1875299.

[12] Martínez-González MÁ, Corella D, Salas-salvadó J, Ros E, Covas MI, Fiol M, et al. Cohort profile: design and methods of the PREDIMED study. Int J Epidemiol 2012;41:377–85. https://doi.org/10.1093/ije/dyq250.
[13] Investigator Tools. The Thematic network - predimed. es n.d, http://www.predimed.es/investigators-tools.html. [Accessed 28 September 2022].
[14] Mayo Clinic Laboratories. CUS1 - overview: copper, serum. n.d, https://www.mayocliniclabs.com/test-catalog/overview/616155#Clinical-and-Interpretive. [Accessed 26 September 2022].
[15] Estruch R, Ros E, Salas-Salvadó J, Covas M-I, Corella D, Arós F, et al. Primary prevention of cardiovascular disease with a mediterranean diet supplemented with extra-virgin olive oil or nuts. N Engl J Med 2018;378:e34. https://doi.org/10.1056/nejmoa1800389.
[16] Schröder H, Fitó M, Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, et al. A Short screener is valid for assessing mediterranean diet adherence among older Spanish men and women. J Nutr 2011;141:1140–5. https://doi.org/10.3945/jn.110.135566.
[17] Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, Toledo E, et al. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br J Nutr 2010;103:1808–16. https://doi.org/10.1017/S0007114509993837.
[18] Mataix J, García L, Mañas M, Martínez ELJ. Tabla de composición de Alimentos. fith ed. Granada: Universidad de Granada; 2009.
[19] Elosua R, Garcia M, Aguilar A, Molina L, Covas M-I, Marrugat J. Validation of the Minnesota leisure time Spanish women. Med Sci Sports Exerc 2000;32:1431–7.
[20] Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota leisure time physical activity questionnaire in Spanish men. Am J Epidemiol 1994;139:1197–209. https://doi.org/10.1093/oxfordjournals.aje.a116966.
[21] Hackshaw A. A concise guide to observational studies in healthcare. first ed. Chichester: John Wiley & Sons Inc; 2015.
[22] Hosmer DW, Lemeshow S, Sturdivant RX. Applied logistic regression. third ed. New YorK: Jhon Wiley & Son; 2013.
[23] Bost M, Houdart S, Oberli M, Kalonji E, Huneau JF, Margaritis I. Dietary copper and human health: current evidence and unresolved issues. J Trace Elem Med Biol 2016;35:107–15. https://doi.org/10.1016/j.jtemb.2016.02.006.
[24] Wapnir RA. Copper absorption and bioavailability. Am J Clin Nutr 1998;67:1054–60. https://doi.org/10.1093/ajcn/67.5.1054S.
[25] Johnson PE, Milne DB, Lykken GI. Effects of age and sex on copper absorption, biological half-life, and status in humans. Am J Clin Nutr 1992;56:917–25. https://doi.org/10.1093/ajcn/56.5.917.
[26] Vahter M, Åkesson A, Lidén C, Ceccatelli S, Berglund M. Gender differences in the disposition and toxicity of metals. Environ Res 2007;104:85–95. https://doi.org/10.1016/j.envres.2006.08.003.
[27] Roychoudhury S, Nath S, Massanyi P, Stawarz R, Kacaniova M, Kolesarova A. Copper-induced changes in reproductive functions: in vivo and in vitro effects. Physiol Res 2016;65:11–22. https://doi.org/10.33549/physiolres.933063.
[28] Milne DB. Copper intake and assessment of copper status. Am J Clin Nutr 1998;67. https://doi.org/10.1093/ajcn/67.5.1041S.
[29] Nan Y, Bai Y. Sex-based differences in the association between serum copper and kidney function: evidence from NHANES 2011–2016. Int J Environ Res Publ Health 2022;19. https://doi.org/10.3390/ijerph192114086.
[30] Karadas S, Sayin R, Aslan M, Gonullu H, Kati C, Dursun R, et al. Serum levels of trace elements and heavy metals in patients with acute hemorrhagic stroke. J Membr Biol 2014;247:175–80. https://doi.org/10.1007/s00232-013-9621-0.
[31] Chen A, Li G, Liu Y. Association between copper levels and myocardial infarction: a meta-analysis. Inhal Toxicol 2015;27:237–46. https://doi.org/10.3109/08958378.2015.1030480.
[32] Zhang M, Li W, Wang Y, Wang T, Ma M, Tian C. Association between the change of serum copper and ischemic stroke: a systematic review and meta-analysis. J Mol Neurosci 2020;70:475–80. https://doi.org/10.1007/s12031-019-01441-6.
[33] Bao QJ, Zhao K, Guo Y, Wu XT, Yang JC, Yang MF. Environmental toxic metal contaminants and risk of stroke: a systematic review and meta-analysis. Environ Sci Pollut Control Ser 2022;29:32545–65. https://doi.org/10.1007/s11356-022-18866-z.
[34] Chowdhury R, Ramond A, O'Keeffe LM, Shahzad S, Kunutsor SK, Muka T, et al. Environmental toxic metal contaminants and risk of cardiovascular disease: systematic review and meta-analysis. Br Med J 2018;362:14–6. https://doi.org/10.1136/bmj.k3310.
[35] Salonen JT, Salonen R, Korpela H, Suntioinen S, Tuomilehto J. Serum copper and the risk of acute myocardial infarction: a prospective population study in men in Eastern Finland. Am J Epidemiol 1991;134:268–76. https://doi.org/10.1093/oxfordjournals.aje.a116080.
[36] Isiozor NM, Kunutsor SK, Voutilainen A, Khan S, Kauhanen J, Laukkanen JA. Serum copper and risk of cardiovascular disease mortality among men without diabetes: a 26-year prospective cohort study. Metabolism 2021;116:154656. https://doi.org/10.1016/j.metabol.2020.154656.
[37] Reunanen A, Knekt P, Marniemi J, Mäki J, Maatela J, Aromaa A. Serum calcium, magnesium, copper and zinc and risk of cardiovascular death. Eur J Clin Nutr 1996;50:431–7.
[38] Cabral M, Kuxhaus O, Eichelmann F, Kopp JF, Alker W, Hackler J, et al. Trace element profile and incidence of type 2 diabetes, cardiovascular disease and colorectal cancer: results from the EPIC-Potsdam cohort study. Eur J Nutr 2021;60:3267–78. https://doi.org/10.1007/s00394-021-02494-3.
[39] Wen Y, Huang S, Zhang Y, Zhang H, Zhou L, Li D, et al. Associations of multiple plasma metals with the risk of ischemic stroke: a case-control study. Environ Int 2019;125:125–34. https://doi.org/10.1016/j.envint.2018.12.037.
[40] Kok FJ, Van Duijn CM, Hofman A, Van Der Voet GB, De Wolff FA, Paays CHC, et al. Serum copper and zinc and the risk of death from cancer and cardiovascular disease. Am J Epidemiol 1988;128:352–9. https://doi.org/10.1093/oxfordjournals.aje.a114975.
[41] Zhang J, Cao J, Zhang H, Jiang C, Lin T, Zhou Z, et al. Plasma copper and the risk of first stroke in hypertensive patients: a nested case-control study. Am J Clin Nutr 2019;110:212–20. https://doi.org/10.1093/ajcn/nqz099.
[42] Palaniswamy S, Piltonen T, Koiranen M, Mazej D, Järvelin MR, Abass K, et al. The association between blood copper concentration and biomarkers related to cardiovascular disease risk – analysis of 206 individuals in the Northern Finland Birth Cohort 1966. J Trace Elem Med Biol 2019;51:12–8. https://doi.org/10.1016/j.jtemb.2018.09.003.
[43] Jeremy JY, Shukla N, Angelini GD, Day A, Wan IYP, Talpahewa SP, et al. Sustained increases of plasma homocysteine, copper, and serum ceruloplasmin after coronary artery bypass grafting. Ann Thorac Surg 2002;74:1553–7. https://doi.org/10.1016/S0003-4975(02)03807-9.
[44] Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. J Clin Invest 1986;77:1370–6. https://doi.org/10.1172/JCI112442.
[45] Rogero MM, Calder PC. Obesity, inflammation, toll-like receptor 4 and fatty acids. Nutrients 2018;10:1–19. https://doi.org/10.3390/nu10040432.