Redox Biology 29 (2020) 101404

Contents lists available at ScienceDirect

Redox Biology

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

Research Paper

Multiple plasma metals, genetic risk and serum C-reactive protein: A metal-metal and gene-metal interaction study

Yu Yuan[a,1], Pinpin Long[a,1], Kang Liu[a], Yang Xiao[a], Shiqi He[a], Jun Li[a,b], Tingting Mo[a], Yiyi Liu[a], Yanqiu Yu[a], Hao Wang[a], Lue Zhou[a], Xuezhen Liu[a], Handong Yang[c], Xiulou Li[c], Xinwen Min[c], Ce Zhang[c], Xiaomin Zhang[a], An Pan[d], Meian He[a], Frank B. Hu[b], Ana Navas-Acien[e], Tangchun Wu[a,*]

[a] Department of Occupational and Environmental Health, Key Laboratory of Environment and Health, Ministry of Education and State Key Laboratory of Environmental Health (Incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[b] Department of Nutrition, and Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, 02115, USA
[c] Department of Cardiovascular Diseases, Sinopharm Dongfeng General Hospital, Hubei University of Medicine, Shiyan, China
[d] Department of Epidemiology and Biostatistics, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[e] Mailman School of Public Health, Columbia University, 722 West 168th Street, New York, NY, 10032, USA

ARTICLE INFO

Keywords:
Copper
Selenium
C-reactive protein
Metal-metal interaction
Gene-metal interaction
Genetic risk score

ABSTRACT

Background: C-reactive protein (CRP) is a well-recognized biomarker of inflammation, which can be used as a predictor of cardiovascular disease. Evidence have suggested exposure to multiple metals/metalloids may affect immune system and give rise to cardiovascular disease. However, it is lack of study to comprehensively evaluate the association of multiple metals and CRP, the interactions between metals, and the gene-metal interaction in relation to CRP levels.

Aims: To explore the associations of multiple plasma metals with serum CRP, and to test the interactions between metals, and gene-metal interactions on the levels of serum CRP.

Methods: We included 2882 participants from the Dongfeng-Tongji cohort, China, and measured 23 plasma metals and serum CRP concentrations. The genetic risk score (GRS) was calculated based on 7 established CRP-associated variants. For metals which were associated with the levels of CRP, we further tested the interactions between metals on CRP, and analyzed the gene-metal interactions on CRP.

Results: The median level for CRP in the total population was 1.17 mg/L. After multivariable adjustment, plasma copper was positively associated with serum CRP ($FDR < 0.001$), whereas selenium was negatively associated with serum CRP ($FDR = 0.01$). Moreover, selenium and zinc attenuated the positive association between high plasma copper and CRP (P for interaction < 0.001). Participants with a higher GRS had a higher CRP level, with the increase in ln-transformed CRP per increment of 5 risk alleles were 0.64 for weighted GRS, and 0.54 for unweighted GRS (both $P < 0.001$). Furthermore, the genetic association with CRP was modified by copper concentration (P for interaction < 0.001).

Conclusions: Our results suggest that serum CRP is positively associated with plasma concentration of copper, and inversely associated with selenium. Plasma zinc, selenium and CRP genetic predisposition would modify the associations between plasma copper and serum CRP.

1. Introduction

With rapid urbanization and industrialization, co-exposure to multiple metals/metalloids from geological origin have become a global public health threat in recent decades [1–3]. Emerging evidence suggest that chronic exposure to metals may affect immune system and give rise to cardiovascular disease [1,3,4]. C-reactive protein (CRP) is a well-recognized acute-phase and nonspecific biomarker of

* Corresponding author. Department of Occupational and Environmental Health, Hubei Key Laboratory of Environment and Health (Incubating), and Ministry of Education and State Key Laboratory of Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong Road, Wuhan, 430030, China.
E-mail address: wut@mails.tjmu.edu.cn (T. Wu).
[1] These authors contributed equally to the study.

https://doi.org/10.1016/j.redox.2019.101404
Received 11 October 2019; Received in revised form 2 December 2019; Accepted 7 December 2019
Available online 10 December 2019

inflammation [5]. It is involved in the immunologic process that triggers vascular remodeling and plaque deposition and it can be used as a predictor of cardiovascular disease [5,6]. A limited number of epidemiological studies have investigated the associations between several metals/metalloids (copper [7–9], zinc [8,10,11], selenium [12,13], magnesium [14,15], lead [16]) and the inflammation marker CRP. These studies, however, had relatively small sample sizes ($n < 200$) [7–10,14], restricted study populations (participants with specific diagnosis, including HIV infection, malaria [8,9,11,14]), or lacked adjustment for potential confounding (including age, sex) [7,8,17]. Furthermore, most existing observations did not fully explore the associations of multiple metals with CRP, or take into account the interactions between metals. People are exposed to multiple metals simultaneously in the real-world scenario, and the interactions among metals occur frequently and can both increase and decrease the toxicity of metals [3].

Recently, a large scale genome-wide association studies (GWAS) have identified 7 single nucleotide polymorphisms (SNPs) that are associated with CRP levels among Asian population [18]. These SNPs play important roles in the biological pathways of regulating CRP levels. As a major source of inflammatory stimuli, exposure to multiple metals may participate in the CRP regulation through related pathways. Investigations of the gene–environment interaction studies are effective tools for assessing hypothesized biological mechanisms and advancing the mechanistic understanding [19,20]. However, despite the importance, to the best of our knowledge, no study has investigated whether CRP genetic variations may modify the associations of multiple metals and CRP.

Therefore, in the current study, we analyzed the associations between exposure to multiple metals and serum CRP. In addition, we calculated the genetic risk score (GRS) on the basis of the 7 loci that have been associated with CRP [21], and particularly examined the interactions between metals and GRS in relation to CRP among the Dongfeng-Tongji cohort population.

2. Methods

2.1. Study population

The Dongfeng-Tongji (DFTJ) cohort is a population-based survey established from September 2008 to June 2010 including 27,009 retired employees from the Dongfeng Motor Corporation (DMC), Shiyan City, China. Trained interviewers administered a standardized questionnaire to collect information on socio-demographic characteristics (age, sex, education level), lifestyle behaviors (alcohol consumption, tobacco smoking, physical activity, dietary intake), personal health and medical history. The participants also completed physical examinations, and provided peripheral venous blood samples after overnight fasting at baseline [22]. In 2013, they were face-to-face interviewed and completed physical examinations again, and the overall follow-up rate was 96.2%. We conducted a nested-case control study including 1621 incident coronary heart disease (CHD) cases and 1621 age and sex-matched controls within this cohort to assess the prospective association between plasma metal concentrations and incident CHD ($n = 3242$) [23]. The eligibility criteria and study design for this study have been described in detail in previous publications [23]. The 1621 cases were those free of CHD, stroke at baseline but developed CHD during the follow-up, whereas the 1621 age and sex-matched controls were those free of CHD, stroke both at baseline and during the follow-up (until 31 December 2013). After the exclusion of participants with missing values for serum CRP or CRP levels > 10 mg/L, a total of 2882 participants with genotyping data were included in the present study. All participants provided written informed consent forms. The study protocol was approved by the Institutional Review Board of the Tongji Medical College, Wuhan, China.

2.2. Assessment of CRP

The blood samples were drawn in the morning after overnight fasting, collected in EDTA tubes, centrifuged and stored at −80 °C before processed in the laboratory tests. Serum CRP was measured by Latex turbidimetric Immunoassay on ABBOTT ARCHITECT C16000 Fully Automatic Biochemical Analyzer using C-reactive Protein Assay Kit (Abbott, Shanghai, China). CRP levels of each case-control pair were measured in the same analytical run in a random sequence, quality control samples were dispersed throughout each analytic run. The limit of detection (LOD) for serum CRP was 0.10 mg/L and only 14 participants (0.4%) were below the LOD. CRP concentrations below the LOD were imputed with a value equal to the half of the LOD (0.05 mg/L). The intra-assay and inter-assay coefficient of variation for serum CRP was 6.5% and 3.4%, respectively.

2.3. Assessment of metals

Plasma metals/metalloids (aluminum, antimony, arsenic, barium, cadmium, chromium, cobalt, copper, iron, lead, manganese, molybdenum, nickel, rubidium, selenium, strontium, thallium, tin, titanium, tungsten, uranium, vanadium, zinc) were measured by Agilent 7700x inductively coupled plasma mass spectrometer (ICP-MS) following a standardized protocol, and the methods of accuracy evaluation and quality control have been described previously [23,24]. We tested the accuracy of ICP-MS by measuring certified reference agents (ClinChek human plasma controls for trace elements no. 8883 and 8884, Recipe, Munich, Germany) and standard reference materials 1640a (Trace Elements in Natural Water, National Institute of Standards and Technology, Gaithersburg, MD) in every 20 plasma biospecimens. Additionally, we measured a spiked mixed specimen of 100 randomly selected plasma samples for metals without standard reference agents (rubidium, titanium and tungsten). The spike recovery values of those three metals ranged from 82.9% to 105.8%. Intra-assay and inter-assay coefficients of variations for all plasma metals were below 10%. We excluded tungsten, tin, and uranium from further analysis given more than half of the participants (98.3%, 80.2%, and 54.0%, respectively) had plasma concentrations < LOD [23]. For all other metals, less than 12% of the samples had values below the LOD, and they were imputed with a value equal to the half of the detection limit. Previously, we have explored the correlations of metals between plasma, whole blood, and urine, and observed poor correlation of plasma chromium, iron, and cadmium with both whole blood and urine concentrations [23]. Moreover, some evidence suggested these metals mostly locate in the blood cells [3,25,26]. Therefore, we assumed plasma cadmium, chromium and iron were not reliable biomarkers to accurately reflect internal exposure, which were excluded from further analyses. Finally, seventeen plasma metals were included in the present study.

2.4. Assessment of covariates

Information on socio-demographics (age, sex, education), lifestyles (smoking status, drinking status), personal medical histories (hypertension, hyperlipidemia, diabetes) were collected from semi-structured questionnaires. Education level was categorized as primary school or below, middle school, or high school or beyond. Current smokers were defined as smoking at least one cigarette per day over the last 6 months, former smokers were participants who quitted smoking for more than six months. Current drinkers were defined as drinking at least one time per week over the last 6 months, former drinkers were participants who quitted drinking for more than six months. Regular exercise was identified as those who regularly exercised > 30 min/day and > 5 days/week. The measurements of weight, height, blood pressures, blood lipids, fasting glucose, and serum creatinine were conducted at Sinopharm Dongfeng General Hospital by well-trained physicians. Body mass index (BMI) was calculated as weight (kg) divided

by the square of height (m^2). Participants were identified as hypertension if they had a self-reported history of hypertension, or self-reported use of anti-hypertensive medications, or with systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg. Participants were identified as having diabetes mellitus if the level of fasting glucose was 7.0 mmol/L or higher, or with a self-reported history of diabetes, or use of anti-diabetic medications. Hyperlipidemia was identified if total cholesterol was ≥5.72 mmol/L, or triglycerides was ≥1.70 mmol/L, or with self-reported physician diagnosis or use of lipid-lowering drugs. The estimated glomerular filtration rate (eGFR) was calculated by the Modification of Diet in Renal Disease equation based on Chinese patients with chronic kidney disease [27].

2.5. Genotyping and calculation of the GRS

We selected seven single nucleotide polymorphisms (SNPs) associated with CRP which were identified from the largest and most recently published genome-wide association study among Japanese individuals (n = 75,391) [18], including rs12133641 in *IL6R*, rs3093068 in *CRP*, rs814295 in *GCKR*, rs79802086 in *LOC401312*, rs1169284 in *HNF1A*, rs151233628 in *WSCD1*, and rs429358 in *APOE*. The genotypes of the selected 7 SNPs were determined from the GWAS scan with Affymetrix Genome-Wide Human SNP Array 6.0 chips and Illumina Infinium OmniZhongHua-8 Chips. The detailed genotyping procedures and quality control processes have been described in detail elsewhere [28,29]. All SNPs satisfy the criteria of the Hardy–Weinberg equilibrium ($P > 0.05/7 = 0.007$) and the minor allele frequency (MAF) > 0.01 in Chinese population.

The GRS was calculated based on the 7 selected CRP associated genetic variants by use of a previously reported weighted method [21]. Each SNP was recoded as 0, 1, or 2 according to the number of risk alleles (CRP increasing alleles). We calculated the unweighted GRS by summation of the number of risk alleles. We calculated the weighted GRS by using the equation: GRS = (β1 × SNP1 + β2 × SNP2 + … + β7 ×SNP7) × (7/sum of the β coefficients), where the β is the β coefficient of each individual SNP on CRP obtained from the above mentioned GWAS study [18], and sum of the β coefficients is 0.496 in the current analysis. The genetic risk score ranges from 0 to 14, with each unit corresponding to one risk allele and higher scores indicating a higher genetic predisposition to CRP levels.

2.6. Statistical analysis

We categorized CRP to low and high groups according to the median concentrations of CRP in the controls (1.02 mg/L). Baseline characteristics of total participants and by two CRP groups are presented as mean (standard deviation, SD) or median (interquartile range, IQR) for continuous variables and number (percentage) for categorical variables. Given the skewed-distributions of CRP and metals, we used natural log-transformed values to achieve approximately symmetrical distributions. To increase comparability of estimates, the metals concentrations were further standardized to z scores before analyses. We assessed the associations of 17 metals with CRP levels through generalized linear regression models with adjustment for age (continuous), sex, smoking status (current/ever/never), drinking status (current/ever/never), body mass index (BMI) (continuous), education (primary school or below/middle school/high school or beyond), hypertension (yes/no), diabetes (yes/no), hyperlipidemia (yes/no), eGFR (continuous), and future disease status of CHD (yes/no). We also conducted restricted cubic splines to visualize the potential non-linear associations of copper and selenium with CRP levels using 3 knots placed at the 5th, 50th, and 95th percentiles of its distribution, with the reference value set at the 10th percentile of metal concentrations. The extreme metal values below the 1st percentile or higher than the 99th percentile were excluded to avoid the influence on the spline. In the sensitivity analysis, we conducted similar analyses among the 1390 participants identified as control participants (free of CHD and stroke at baseline survey and during follow-up) of the original CHD nested case-control study to exclude the potential influence of the future case-status of CHD. After deriving *P* values for each individual metal, we also derived corresponding *P* values after False Discovery Rate (*FDR*) adjustment (using published software [30]). Two-sided *FDR* < 0.05 was regarded as statistically significant. Furthermore, we evaluated metal-CRP associations among high or low CRP subgroups. Stratified analyses were also performed according to baseline characteristics, including age (< 65, ≥65 years), sex, BMI (< 24.0, ≥24.0 kg/m^2), presence of hypertension, diabetes, and hyperlipidemia (yes, no) in the generalized linear model.

We further evaluated the interactions between metals for the metals which have significant associations with CRP after *FDR* adjustment in the fully adjusted model (copper-selenium), or metals with evidence of related biological mechanisms on CRP (copper-zinc) [9,31]. We divided the individual metals into low, median, and high groups based on tertiles among the participants identified as control subjects in the original nested case-control study. Therefore, a combined category variable was created for two metals. We calculated the multivariable-adjusted mean values of CRP concentrations of the nine groups. *P* for interactions were calculated by including the product term of the metal concentrations (both continuous variables) in the multivariable-adjusted model.

The associations between SNP genotypes and serum CRP levels were evaluated using a linear regression model assuming additive effects of the alleles (0, 1 and 2). The SNPTEST software package was used to perform this statistical analysis (Wellcome Trust Case Control 2007). We performed principal components analysis (PCA) to correct for the population stratification using EIGENSTRAT software [32], and adjusted for age, sex and the top three eigenvectors (the principal components) in the linear regression models. We extracted the results of seven selected SNPs from the genome-wide association analysis.

We then evaluated whether the CRP-GRS (weighted and unweighted GRS) and each CRP related SNP would modify the associations of copper and selenium on CRP. The weighted and unweighted genetic risk scores were divided into three groups (low, median, high) by tertiles of the total population. Generalized linear model was used to estimate the differences in CRP levels according to tertiles of the genetic risk score. We also examined the differences in CRP per increment of 5-risk allele. We calculated the multivariable-adjusted mean values of CRP concentrations according to the combined categories of metal and GRS. Interaction effects between metals and genetic risk scores with respect to CRP levels were tested by modeling an interaction term between each metal (continuous) and GRS (continuous) in generalized linear regression models, adjusted for age, sex, BMI, smoking status, drinking status, education, hypertension, diabetes, hyperlipidemia, eGFR, and future disease status of CHD. We further estimated the differences in ln-transformed CRP levels associated with a 1-standard deviation increase in ln-transformed metals concentrations according to each CRP-related gene variant genotypes using generalized linear model.

For all associations, we reported beta coefficients (β) and their 95% confidence intervals (CIs) which represent the estimated difference in ln-transformed dependent variables associated with a 1-SD increase in ln-transformed independent variables. We performed analyses with SAS version 9.3 (SAS Institute, Cary, NC) and R software (version 3.1.2, R Core Team), and defined statistical significance as two-tailed $P < 0.05$. Power calculations were performed with GPower 3.1.9.4 [33] for GRS-metal interactions, and Quanto1.2.4 (http://biostats.usc.edu/Quanto.html) for SNP-metal interactions.

3. Results

3.1. General characteristics of the study population

The baseline characteristics of the total study participants

Table 1
Baseline characteristics of study participants.

Characteristics [a]	Low CRP group (n = 1282) [b]	High CRP group (n = 1600)	Total participants (n = 2882)
Age, years	64.90 ± 7.34	66.12 ± 7.14	65.58 ± 7.25
Male, n (%)	633 (49.4)	777 (48.6)	1410 (48.9)
BMI, kg/m^2	23.73 ± 3.07	25.26 ± 3.27	24.58 ± 3.27
Smoking status, n (%)			
Current smoker	239 (18.6)	338 (21.1)	577 (20.0)
Former smoker	157 (12.3)	184 (11.5)	341 (11.8)
Never smoker	886 (68.1)	1078 (67.4)	1964 (68.2)
Drinking status, n (%)			
Current drinker	291 (22.7)	356 (22.3)	647 (22.5)
Former drinker	64 (5.0)	72 (4.5)	136 (4.7)
Never drinker	927 (72.3)	1172 (73.2)	2099 (72.8)
Education level, n (%)			
Primary school or below	399 (31.1)	547 (34.2)	946 (32.8)
Middle school	454 (35.4)	578 (36.1)	1032(35.8)
High school or beyond	429 (33.5)	475 (29.7)	904 (31.4)
Hypertension, n (%)	612 (47.7)	983 (61.4)	1595 (55.3)
Dyslipidemia, n (%)	607 (47.3)	925 (57.8)	1532 (53.2)
Diabetes, n (%)	183 (14.3)	316 (19.8)	499 (17.3)
eGFR, mL/min/1.73 m^2	84.89 (74.37, 99.58)	84.14 (72.48, 96.04)	84.56 (73.34, 97.39)
C-reactive protein, mg/L	0.54 (0.34, 0.74)	2.20 (1.51, 3.60)	1.17 (0.59, 2.40)
Metals, μg/L			
Aluminum	54.17 (32.40, 123.21)	53.16 (32.11, 117.60)	53.43 (32.26, 118.62)
Antimony	0.14 (0.09, 0.22)	0.14 (0.09, 0.22)	0.14 (0.09, 0.22)
Arsenic	2.12 (1.36, 4.05)	2.12 (1.36, 4.06)	2.13 (1.36, 4.05)
Barium	38.77 (24.14, 68.55)	37.63 (24.08, 67.85)	38.18 (24.10, 68.19)
Cobalt	0.16 (0.12, 0.20)	0.15 (0.12, 0.20)	0.15 (0.12, 0.20)
Copper	903.0 (798.4, 1005.6)	1001.67 (900.29, 1128.93)	959.89 (854.27, 1072.48)
Lead	13.52 (9.21, 23.09)	13.58 (9.39, 21.15)	13.55 (9.32, 22.12)
Manganese	4.10 (3.05, 5.67)	4.20 (3.14, 5.87)	4.17 (3.08, 5.80)
Molybdenum	1.38 (1.10, 1.74)	1.34 (1.10, 1.76)	1.36 (1.10, 1.75)
Nickel	2.98 (2.12, 4.41)	3.04 (2.23, 4.51)	3.02 (2.18, 4.49)
Rubidium	356.00 (318.00, 398.50)	356.03 (317.77, 401.10)	356.03 (317.81, 399.85)
Selenium	67.32 (57.70, 77.54)	65.87 (56.98, 76.72)	66.50 (57.34, 77.29)
Strontium	36.08 (30.06, 42.99)	36.45 (30.49, 43.22)	36.29 (30.31, 43.11)
Thallium	0.14 (0.10, 0.19)	0.14 (0.10, 0.18)	0.14 (0.10, 0.19)
Titanium	29.55 (24.92, 35.82)	30.19 (24.90, 37.04)	29.88 (24.91, 36.39)
Vanadium	0.69 (0.54, 1.01)	0.68 (0.54, 1.00)	0.68 (0.54, 1.00)
Zinc	1237.50 (1037.50, 2959.00)	1213.49 (1029.85, 2785.50)	1225.04 (1032.49, 2883.11)
Weighted GRS (7 SNPs)	5.73 (4.91, 6.46)	6.15 (5.22, 7.23)	5.87 (4.98, 6.89)
Unweighted GRS (7 SNPs)	6.00 (5.00, 7.00)	6.00 (5.00, 7.00)	6.00 (5.00, 7.00)

Abbreviations: BMI, body mass index; CRP, C-Reactive Protein; eGFR, estimated glomerular filtration rate; GRS, genetic risk score; SNP, single nucleotide polymorphisms.

[a] Data were presented as means (SD) for normally distributed variables, median (IQR) for non-normally distributed variables and numbers (percentages) for categorical variables.

[b] The CRP group (low or high group) were defined by concentrations lower or higher than the median concentration among the participants identified as control subjects in the nested case-control study (1.02 mg/L).

(n = 2882) and the participants in low/high CRP groups are presented in Table 1. In the total study population, the mean age was 65.58 years, and approximately half of the participants were males (48.9%). The study participants had an average BMI of 24.58, and the percentages of current smokers and current drinkers were 20.0% (n = 577) and 22.5% (n = 647), respectively. About 904 (31.4%) of the participants had an education level of high school or beyond. With respect to disease history, there were 1595 (55.3%), 499 (17.3%), and 1532 (53.2%) participants identified as hypertension, diabetes mellitus, and hyperlipidemia. The median concentrations of CRP in total participants and control subjects in the nested case-control study were 1.17 and 1.02 mg/L, respectively. The median values (IQR) of the weighted and unweighted genetic risk score were 5.87 (4.98, 6.89) and 6.00 (5.00, 7.00), respectively.

3.2. Associations of plasma metals with serum CRP

Plasma copper was positively associated with serum CRP levels, while plasma selenium and plasma molybdenum were inversely associated with serum CRP levels with adjustment for age and sex in model 1 (all *FDR* < 0.05) (Table 2). Other metals/metalloids were not associated with serum CRP. In the fully adjusted model (model 2), the linear associations of copper and selenium with CRP remained significant (all *FDR* < 0.05), and the adjusted beta coefficients (95% CI) were 0.262 (0.227, 0.296) and −0.058 (−0.093, −0.022), respectively. The restricted cubic splines demonstrated significant non-linear relation between copper and CRP, with a slight decrease in slope for copper concentrations above 960 μg/L (*P* for non-linear association = 0.03, Fig. 1A). On the contrary, we did not find significant non-linear relation for selenium and CRP (*P* for non-linear association = 0.63, Fig. 1B). The sensitivity analyses conducted in the control subjects showed similar results to the primary results for copper and CRP, however, the association between selenium and CRP was not statistically significant after *FDR* adjustment (*FDR* = 0.06) (Table S1).

When the analysis was stratified to compare subgroups of participants (Fig. S1) with serum CRP values of ≤1.02 (low CRP group) and > 1.02 mg/L (high CRP group), a significant inverse association of plasma selenium with CRP was observed in the high group (−0.05, 95% CI: 0.08, −0.02, *P* < 0.001), but not in the low group (0.001, 95% CI: 0.03, 0.03, *P* = 0.96). Plasma copper was significantly associated with CRP levels in both high and low CRP groups (both *P* < 0.001) (0.07 vs. 0.19). The associations of CRP with copper and

Table 2
Estimated difference in ln-transformed C-reactive protein levels [β (95% CI)] associated with a 1-standard deviation increase in ln-transformed exposure levels of metals using generalized linear regression models (n = 2882).

Metals	Model 1[a]			Model 2[b]		
	β (95% CI)	*P* Value	*FDR*	β (95% CI)	*P* Value	*FDR*
Aluminum	0.018 (−0.018, 0.054)	0.33	0.57	0.003 (−0.032, 0.038)	0.87	0.93
Antimony	0.018 (−0.019, 0.054)	0.34	0.57	0.020 (−0.015, 0.055)	0.26	0.62
Arsenic	0.012 (−0.024, 0.048)	0.52	0.68	−0.010 (−0.045, 0.025)	0.59	0.77
Barium	0.002 (−0.034, 0.038)	0.90	0.90	−0.011 (−0.046, 0.023)	0.52	0.77
Cobalt	−0.031 (−0.068, 0.005)	0.09	0.25	−0.023 (−0.057, 0.012)	0.19	0.62
Copper	**0.258 (0.222, 0.294)**	**< 0.001**	**< 0.001**	**0.262 (0.227, 0.296)**	**< 0.001**	**< 0.001**
Lead	0.008 (−0.028, 0.045)	0.65	0.79	−0.002 (−0.036, 0.033)	0.93	0.93
Manganese	0.037 (0.0002, 0.073)	0.05	0.19	0.038 (0.003, 0.072)	0.03	0.19
Molybdenum	−0.038 (−0.074, −0.001)	0.04	0.19	−0.013 (−0.048, 0.023)	0.48	0.77
Nickel	0.014 (−0.022, 0.051)	0.44	0.66	0.021 (−0.014, 0.055)	0.24	0.62
Rubidium	−0.020 (−0.057, 0.017)	0.29	0.57	−0.031 (−0.067, 0.004)	0.08	0.34
Selenium	**−0.058 (-0.094, -0.021)**	**0.002**	**0.02**	**−0.058 (-0.093, -0.022)**	**0.001**	**0.01**
Strontium	0.035 (−0.001, 0.071)	0.06	0.19	0.017 (−0.018, 0.052)	0.34	0.73
Thallium	−0.006 (−0.042, 0.031)	0.76	0.82	−0.014 (−0.049, 0.021)	0.43	0.77
Titanium	0.019 (−0.017, 0.055)	0.30	0.57	0.005 (−0.029, 0.040)	0.76	0.86
Vanadium	0.013 (−0.023, 0.050)	0.46	0.66	0.006 (−0.028, 0.041)	0.72	0.86
Zinc	0.005 (−0.031, 0.042)	0.77	0.82	−0.010 (−0.045, 0.025)	0.57	0.77

[a] Model 1 was adjusted for age and sex.
[b] Model 2 was adjusted for age, sex, body mass index, smoking status, drinking status, education, hypertension, diabetes, hyperlipidemia, estimated glomerular filtration rate, and the future disease status of CHD.

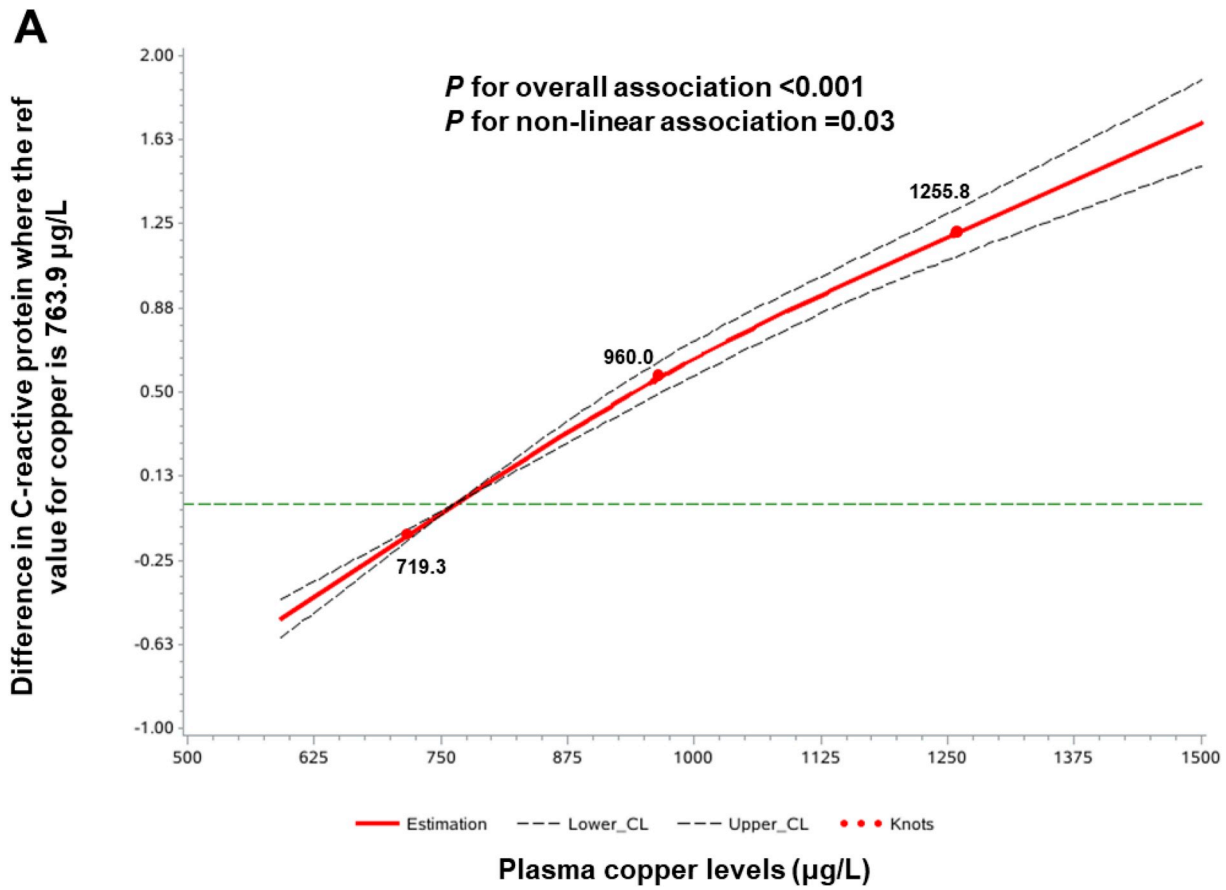


Fig. 1A. Adjusted dose-response association between plasma copper and serum C-reactive protein. Knots were placed at the 5th (719.3 µg/L), 50th (960.0 µg/L), and 95th (1255.8 µg/L) percentiles of the plasma copper distribution, and the reference value was set at the 10th percentile (763.9 µg/L). Y-axis represents the difference in serum C-reactive protein levels between individuals with any value of plasma copper with individuals with 763.9 µg/L of plasma copper. Dashed lines are 95% confidence intervals. Adjustment factors were age, sex, body mass index, smoking status, drinking status, education, hypertension, diabetes, hyperlipidemia, estimated glomerular filtration rate, and the future disease status of CHD.

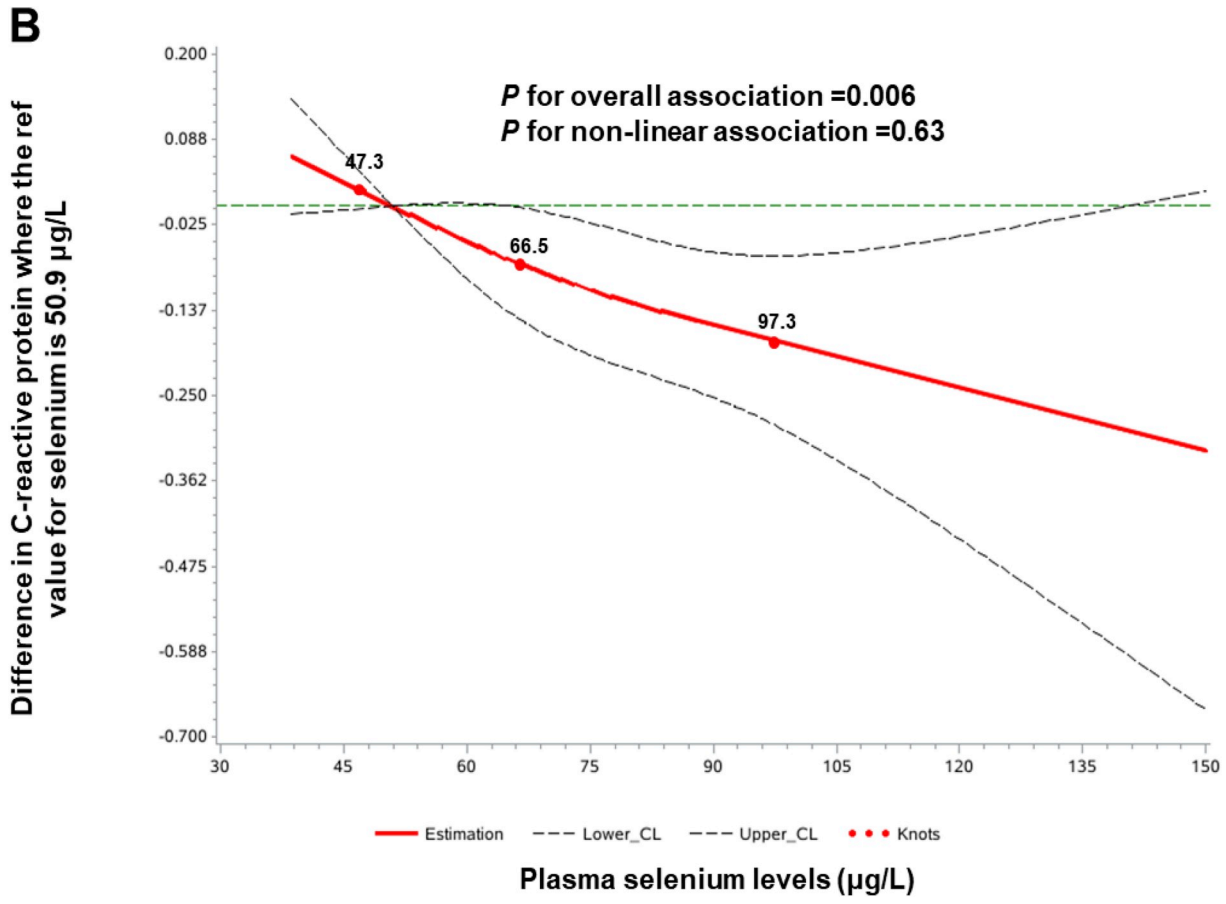
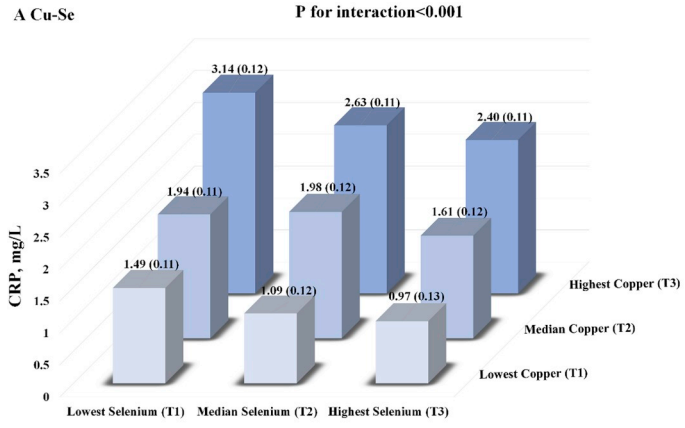
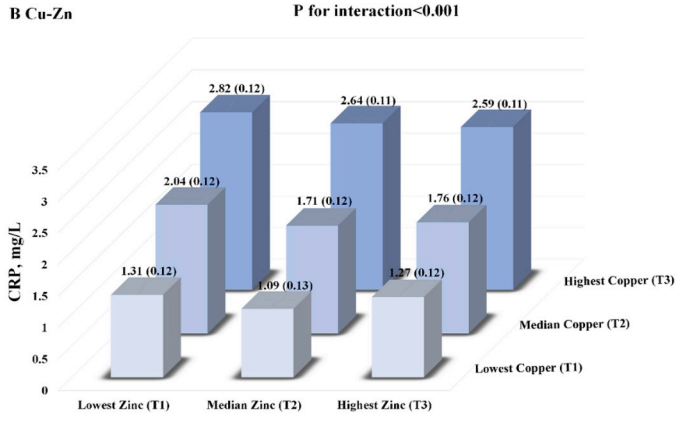


Fig. 1B. Adjusted dose-response association between plasma selenium and serum C-reactive protein. Knots were placed at the 5th (47.3 μg/L), 50th (66.5 μg/L), and 95th (97.3 μg/L) percentiles of the of plasma selenium distribution, and the reference value was set at the 10th percentile (50.9 μg/L). Y-axis represents the difference in serum C-reactive protein levels between individuals with any value of plasma selenium with individuals with 50.9 μg/L of plasma selenium. Dashed lines are 95% confidence intervals. Adjustment factors were age, sex, body mass index, smoking status, drinking status, education, hypertension, diabetes, hyperlipidemia, estimated glomerular filtration rate, and the future disease status of CHD.



No. of participants

	Lowest Selenium (T1)	Median Selenium (T2)	Highest Selenium (T3)
Highest Copper (T3)	287	337	421
Median Copper (T2)	336	301	298
Lowest Copper (T1)	383	293	226



No. of participants

	Lowest Zinc (T1)	Median Zinc (T2)	Highest Zinc (T3)
Highest Copper (T3)	288	397	360
Median Copper (T2)	309	303	323
Lowest Copper (T1)	294	286	322

Fig. 2. Concentrations of serum C-reactive protein according to plasma copper, selenium, and zinc status (panel A, copper-selenium; panel B, copper-zinc, n = 2882). Bars are means (SEs) after adjustment of age, sex, body mass index, smoking status, drinking status, education, hypertension, diabetes, hyperlipidemia, estimated glomerular filtration rate, and the future disease status of CHD. The plasma copper, selenium and zinc groups (lowest, median or highest group) were defined by tertiles among the participants identified as control subjects in the original nested case-control study. *P* for interactions were calculated by including the product term of the metal concentrations (continuous variables) in the multivariable-adjusted model.

Table 3
The estimated difference in ln-transformed C-reactive protein levels [β (SE)] associated with tertiles of GRS, and per increment of five risk alleles (n = 2882).

	Tertiles of genetic risk score			*P* trend	Per increment of five risk alleles	
	T1	T2	T3		β (SE)	*P* value
Weighted GRS						
No. of Participants	928	999	955		2882	
Model 1[a]	0 (reference)	0.21 (0.04)	0.43 (0.04)	< 0.001	0.66 (0.06)	< 0.001
Model 2[b]	0 (reference)	0.24 (0.04)	0.41 (0.04)	< 0.001	0.64 (0.06)	< 0.001
Unweighted GRS	< 0.08	0.08–0.13	0.13–0.21			
No. of Participants	1702	660	520		2882	
Model 1	0 (reference)	0.22 (0.04)	0.40 (0.05)	< 0.001	0.56 (0.06)	< 0.001
Model 2	0 (reference)	0.24 (0.04)	0.37 (0.05)	< 0.001	0.54 (0.06)	< 0.001

[a] Model 1 was adjusted for age, sex.
[b] Model 2 was additionally adjusted for body mass index, smoking status, drinking status, education, hypertension, diabetes, hyperlipidemia, estimated glomerular filtration rate, and the future disease status of CHD.

selenium did not appear to be modified by age, sex, BMI, hypertension, diabetes, and hyperlipidemia (Fig. S2).

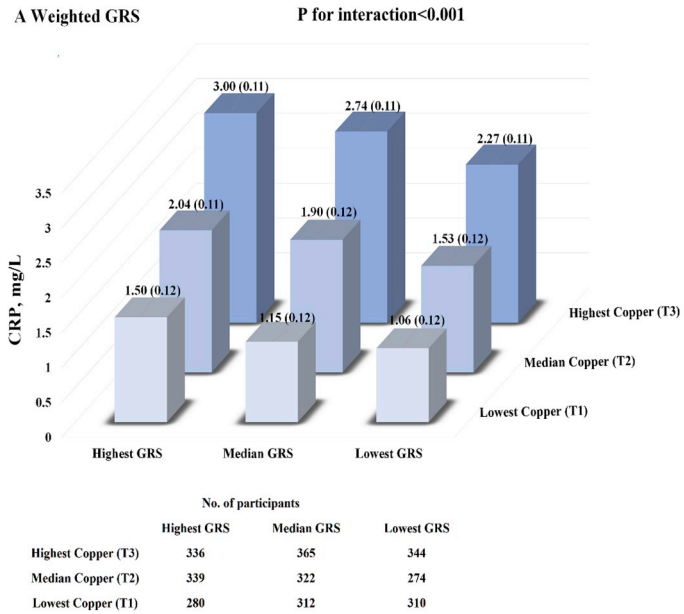
3.3. Interaction between metals on CRP

In Fig. 2, we found significant interactions between copper and selenium, copper and zinc, with respect to serum CRP levels (all *P* for interactions < 0.001), and suggested that higher plasma selenium and zinc concentrations may attenuate the positive association between plasma copper and CRP. Among the nine combined categories created for copper and selenium, the highest multivariable-adjusted mean (SE) of CRP levels was 3.14 (0.12) mg/L in lowest Se/highest Cu group, and the lowest multivariable-adjusted mean (SE) of CRP levels was 0.97 (0.13) mg/L in highest Se/lowest Cu group. Similar interaction patterns were also observed for plasma copper and plasma zinc.

3.4. Interaction between metals and genetic polymorphisms on CRP

Table S2 presents the detailed information of the seven CRP-related SNPs in DFTJ cohort and the published study. The 7-SNP weighted and unweighted GRS explained 3.3% and 2.5% of the variation in CRP among our study participants, respectively. The estimated difference in CRP levels were gradually increased across tertiles of weighted and unweighted GRS (all *P* for trend < 0.001). After multivariable adjustment, differences in ln-CRP per 5-risk allele increment were 0.64 (SE, 0.06) and 0.54 (SE, 0.06) for weighted and unweighted GRS, respectively (Table 3). Fig. 3 shows multivariable-adjusted CRP levels according to the combined categories of copper status and the genetic risk score. CRP levels were higher with elevated plasma copper concentrations among participants at higher genetic risk, and were lower with decreased plasma copper concentrations among participants at lower genetic risk (both *P* for interaction < 0.001). The adjusted mean (SE) of CRP was 3.00 (0.11) mg/L among participants with highest copper concentration and highest weighted GRS, whereas 1.06 (0.12) mg/L for the lowest copper and lowest GRS group. Similar trend was observed for the combined groups of unweighted GRS and copper concentration. We did not observe significant interactions between CRP-GRS and plasma selenium in relation to CRP levels (data not shown).

The interactions of copper, selenium with each genetic polymorphism in relation to CRP are shown in Table S3. When stratified by



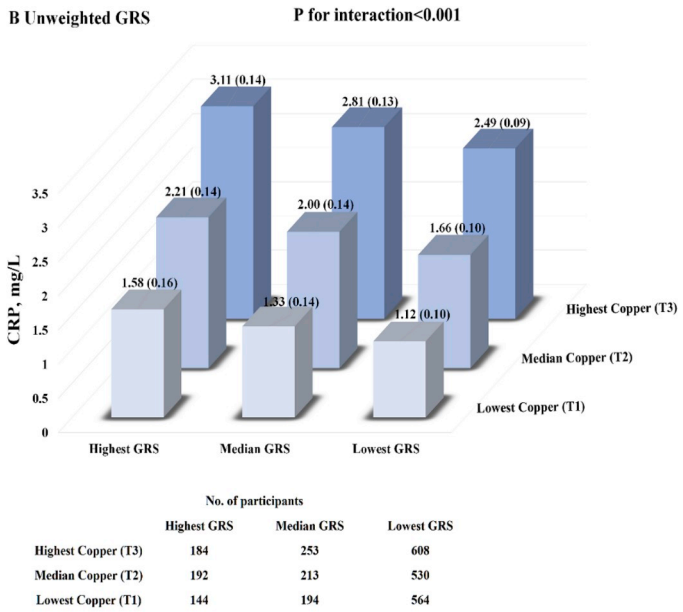


Fig. 3. Concentrations of serum C-reactive protein according to plasma copper status and genetic risk score (GRS, panel A, weighted genetic risk score; panel B, unweighted genetic risk score, n = 2882). Bars are means (SEs) after adjustment of age, sex, body mass index, smoking status, drinking status, education, hypertension, diabetes, hyperlipidemia, estimated glomerular filtration rate, and the future disease status of CHD. The plasma copper groups (lowest, median or highest group) were defined by tertiles among the participants identified as control subjects in the original nested case-control study. The GRS (lowest, median or highest group) groups were defined by tertiles of the total population. *P* for interactions were calculated by including the product term of the copper concentrations with GRS in the multivariable-adjusted model.

SNP genotypes, carriers of rs12133641-GG, rs3093068-GG, rs814295-GG, rs79802086-TT, rs1169284-CC, rs151233628-CT/TT, and rs429358-TT genotype showed stronger associations between copper and CRP (all P for interactions < 0.05). However, we found no interactions between genetic polymorphisms and plasma selenium (all P for interaction > 0.05). The present study had more than 99% statistical power to detect the interaction between CRP-GRS and plasma copper on serum CRP levels. Usually, the statistical power to detect the relatively small effects conferred by each SNP is limited. However, the study still had 70.0%–99.9% power to detect significant ($P < 0.05$) gene-copper interaction effect sizes of 1.17 mg/L for CRP.

4. Discussion

In the present study, serum CRP levels were positively associated with plasma copper concentrations but inversely associated with plasma selenium concentrations, with a monotonic dose-response. Other plasma metals/metalloids were not associated with serum CRP levels. We examined interactions between metals and found that higher selenium and zinc plasma concentrations attenuated the positive association between high plasma copper and CRP. Importantly, we observed consistent associations of CRP genetic score (weighted and unweighted) with the level of CRP. We also found significant interaction between copper and GRS in predicting CRP levels. These results for the first time suggest that individuals with a greater genetic predisposition to CRP might be more susceptible to the adverse influence of copper exposure on CRP. Meanwhile, the copper exposure might magnify genetic effects on CRP.

Copper and zinc are trace elements essential for life and constitute components of about 40 metalloenzymes, including Cu/Zn superoxide dismutase (SOD1) with antioxidant and anti-inflammatory activity [31,34,35]. In the present study, we found positive association between plasma copper with serum CRP. Consistent with our finding, some cross-sectional studies conducted among healthy school children (n = 643, Bui et al., 2012), children with malaria (n = 105, Saad et al., 2013), and schizophrenia case-control pairs (n = 80, Devanarayanan et al., 2016) found positive associations between copper level and serum CRP. Limitations of the existing studies include the restriction to participants of certain diseases in some studies [7,8], the relatively small sample size [7,8,17], and the lack of adjustment for potential confounding factors (including age, sex) [7,8,17].

Zinc affects multiple aspects of the immune system (including the antioxidant property, Prasad 2008), whereas excess zinc can also be toxic [35]. Both a deficiency and an excess of Cu or Zn can cause harm, so the homeostasis of both elements is strictly regulated [31,36,37]. On the one hand, Cu/Zn superoxide dismutase is an important scavenger protein that acts against oxidative stress mediated by reactive oxygen species (ROS) [38]. Meanwhile, zinc could compete with copper for binding to cell membrane and decreasing the production of hydroxyl radical (one type of ROS) [39]. An excess of zinc could also induce copper deficiency through reduction of gene expression related to copper absorption [35]. Despite these evidences, it is lack of epidemiological studies evaluating the interaction of copper and zinc on serum CRP. Our study found that zinc could attenuate the positive association between high plasma copper and CRP. These findings emphasize the importance to monitor the copper and zinc concentrations and maintain a copper-zinc equilibrium, given the toxicity of excess copper is generally not considered to be a widespread health concern [35].

Selenium is incorporated into selenoproteins that have a wide range of effects, ranging from antioxidant and anti-inflammatory effects to the establishment of an effective immune response [40]. In the present study, we found plasma selenium levels were associated with the decreased concentrations of serum CRP. Particularly, serum selenium was associated with decreased CRP levels among individuals with low-grade inflammation (CRP levels $>$ 1.02 mg/L) rather than individuals without inflammation (CRP levels $\leq$ 1.02 mg/L), suggesting the potential beneficial role of selenium in the management of low-grade systemic inflammation. The inverse association between selenium and CRP is in line with the cross-sectional studies of National Health and Nutrition Examination Survey III (blood selenium) [41] and the MONICA/KORA Augsburg Survey (selenium supplementation) [42]. Consistent with these observational findings, a clinical trial among type 2 diabetes or coronary heart disease patients (n = 60) showed that 200 μg/day selenium supplementation would lead to a significant decrease in serum CRP levels [43]. By contrast, 60 μg/day selenium supplementation showed no changes in CRP levels in a clinical trial of 20 patients diagnosed with cancer or sepsis [44]. The CARDIA cohort of 4,032 Americans also found no significant associations between toenail selenium and inflammation as measured by CRP [13]. Various reasons could explain the disparate findings between studies. First, selenium might show a protective effect only over a particular range of status, neither too low nor too high [40]. Therefore, if the selenium level in the reference group was high enough to provide full antioxidant benefits, further selenium intake might not offer any additional benefit [13]. Second, CARDIA used toenail selenium as a biomarker, which may reflect a different exposure time frame and selenium species and internal dose compartment than circulating selenium [13]. Third, the small sample size of one the clinical trial with null findings (n = 20) may limit statistical power [44].

Although an *in vitro* study supported that selenium can inhibit copper-mediated oxidative DNA damage by organoselenium compounds, selenocystine, selenomethionine, and methylselenocysteine due to the metal-binding mechanism [45], no data is available on whether selenium interacts with copper on human immunity. Therefore, we investigated the interactions of copper and selenium on CRP levels, and found exposure to high levels of selenium appeared to attenuate the positive associations between high-plasma copper and CRP. CRP levels < 1, 1 to 3, and > 3 mg/L have been related to lower, average, and higher cardiovascular risk, respectively [46]. Given the relatively high CRP levels (adjusted mean 5.31 mg/L) among participants with low selenium and high copper concentration, we can speculate that these participants are also at higher cardiovascular risk. Some animal studies suggested copper exposure would trigger oxidative stress and inflammation response [47,48]. One major mechanism of copper-induced oxidative stress is through the interaction between Cu^{2+} ions and reduced glutathione (GSH) [49]. Recognized as an important selenoprotein (constituted by selenium), glutathione belongs to the family of antioxidant enzymes that can remove hydrogen peroxide [40], which may play an antagonistic effect. Further investigations are warranted to confirm whether selenium may attenuate adverse relationship of copper on CRP levels, and to investigate potential underlying mechanisms and the possibility of public health interventions.

One of the novel findings of the present study is that the high copper concentration magnifies the genetic predisposition to increased CRP level. We firstly calculated a genetic risk score based on multiple genetic loci, which has become the preferred method in analyses of gene–environment interactions [50,51]. Interestingly, we found a significant interaction between copper exposure and genetic-predisposition score (both weighted and unweighted) in relation to CRP concentration. In addition, we found each of the individual SNPs also show significant interactions with copper concentrations in relation to CRP level. The consistent results from each individual SNP enhanced the robustness of our findings. Our data suggest that the genetic effects on CRP are stronger in persons with higher copper than in those with lower copper concentration, which provides useful information on the role of copper exposure in triggering inflammation. From another perspective, genetic susceptibility may partly account for the diverse response in inflammation in response to copper exposure.

The current finding is in consistent with the study hypothesis of “differential susceptibility”, indicating that vulnerability gene variants may play a role like plasticity genes [52–54]. The genetic risk could

either be magnified by an adverse environment (high copper concentration) or attenuated by a favorable environment (low copper concentration) [52–54]. However, the mechanisms involved in the interaction between copper exposure and the genetic predisposition to CRP remain unclear. Nevertheless, our findings are biologically plausible. GWAS studies have identified several genetic loci that affect serum CRP levels including those in *CRP* gene itself [55], *APOE* gene [56], and *IL6R* gene [18]. *APOE* gene plays a pivotal role in lipoprotein metabolism in the brain and in the periphery, with implications both in Alzheimer's disease and ischemic heart disease [57]. A genetic link between lipid metabolism and inflammation has been suggested by the association between variation in the *APOE* gene and plasma CRP [58]. Excessive copper would participate in ROS generation, increase oxidative damage to lipids, and induce inflammation [35]. It is plausible that some *CRP* and *APOE* gene variants may accelerate lipid oxidation and inflammation processes of copper through their effects on gene expression. Additionally, copper was reported to interact with Apolipoprotein E in Alzheimer's disease [59]. Patients with Alzheimer's disease may have altered copper status, which would affect gene expression and transportation of apolipoproteins, including APOE [59]. *IL6R* polymorphisms have been linked with IL-6 levels and CRP levels [60]. As IL-6 is a precursor of CRP [61], larger stressor-evoked IL-6 responses over time may cause heightened systemic levels of CRP [62]. Experimental study based on rat cardiac cells suggested that copper seemed to induce IL-6 release through enhancing signaling pathways that normally regulate the basal IL-6 release [63]. Copper and *IL6R* variant may interact through increase of levels of IL-6, and so as to increase levels of CRP. It is possible that genes involved in the regulation of lipid oxidation and inflammation (including *CRP*, *APOE*, and *IL6R*) could be underlying the observed interaction, but we could not exclude the involvement of other plausible biological pathways. Rather than any single variant, the interaction on CRP might reflect the cumulative effects of multiple genetic loci. Because direct evidence is limited, further studies are warranted to unravel the biological basis underlying the observed interaction between the CRP–related GRS and copper concentration.

To the best of our knowledge, the present study is among the first to provide evidence of the interactions between metals, and metal exposure may interplay with overall genetic susceptibility on CRP level. We used the comprehensive coverage of the established CRP-associated genetic variants for GRS calculation among Asian population. More importantly, the consistent findings of the interaction between copper exposure and each individual SNP indicate the robustness of our results. Our findings provide new insights into the role of the exposure of multiple metals/metalloids and genetic variation in the regulation of CRP levels. The large sample size provides a decent chance of detecting moderate metal-metal, and gene-metal interactions. Nevertheless, we acknowledge that the current study is exploratory and several limitations need to be considered when interpreting our findings. First, the cross-sectional study design hinders us to draw inferences regarding temporality of the associations. Second, as each metal has unique distributions in the circulation system and different organs, plasma metals may not be optimal biomarkers of internal dose for all metals. In our previous study [24], we found significant correlations between plasma and blood concentrations for copper and selenium, and between plasma and urine concentrations for selenium and zinc. Third, we only measured plasma metals at one time point, which may not reflect chronic exposures for all metals. We have evaluated the reproducibility of plasma metals concentrations collected 5 years apart [24], and detected good reproducibility for plasma copper (intraclass correlation coefficients, ICC = 0.74) and selenium (ICC = 0.64). Although our GRS captured the combined information from the established CRP associated variants to date, it accounts for only a small amount of individual variation in CRP (weighted GRS 3.3% and unweighted GRS 2.5%), which were comparative to a previous study (4-SNP GRS explained 0.8%–2.4%, 18-SNP GRS explained 2.1%–10.8% among populations of different ancestry) [64]. It remains to be explored whether our findings could be generalized to other ethnic groups. Finally, replication studies in other populations and functional studies are warranted to confirm the findings and investigate the expression of the identified genes and verify the suggested biological pathways.

5. Conclusion

In conclusion, we found serum CRP levels were positively associated with plasma concentrations of copper, and inversely associated with plasma selenium. Importantly, the positive association of plasma copper with serum CRP appeared to be attenuated with high plasma zinc and selenium, providing the possibility of future public health interventions. Moreover, our results indicated that the association between copper exposure and CRP level might vary according to differences in genetic predisposition; and, vice versa, the genetic influences on CRP might be modified by copper concentrations. These novel findings provide the potential to bring new insights to personalized prevention and interventions for inflammation and cardiovascular disease, and further emphasize the importance of preventing high concentration of copper in the prevention of inflammation, particularly among those with high genetic predisposition.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Contribution statement

Y.Y. and P.L. contributed to the statistical analysis and interpretation of the results and wrote the manuscript. Y.Y., P.L., K.L., Y.X., S.H., Y.L. and Y.Y. contributed to the acquisition of data and researched the data. Y.Y., P.L. and J.L. contributed to the study design. T.M., H.W., L.Z., X.L., H.Y., X.L., X.M. and C.Z. contributed to the discussion of the project. T.M., X.Z., A.P., M.H., F.H., A.N. and T.W. reviewed and edited the manuscript. T.W. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Funding

This work was supported by the Foundation of the National Key Research and Development Program of China (2016YFC0900800), the National Natural Science Foundation of China (81930092, 91643202, 91843302, and 81390542), the Fundamental Research Funds for the Central Universities (2019kfyXMBZ015), the China Postdoctoral Science Foundation (2018M642858).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

We thank all study participants, research staffs, and students who participated in this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2019.101404.

References

[1] R. Chowdhury, A. Ramond, L.M. O'Keeffe, S. Shahzad, S.K. Kunutsor, T. Muka, et al., Environmental toxic metal contaminants and risk of cardiovascular disease: systematic review and meta-analysis, BMJ 362 (2018) k3310.

[2] D.F. Kapraun, J.F. Wambaugh, C.L. Ring, R. Tornero-Velez, R.W. Setzer, A method for identifying prevalent chemical combinations in the U.S. population, Environ. Health Perspect. 125 (2017) 087017.

[3] G.F. Nordberg, B.A. Fowler, M. Nordberg, Handbook on the Toxicology of Metals, fourth ed., Academic Press, Burlington, 2014.

[4] M. Tellez-Plaza, E. Guallar, A. Navas-Acien, Environmental metals and cardiovascular disease, BMJ 362 (2018) k3435.

[5] S. Kaptoge, E. Di Angelantonio, G. Lowe, M.B. Pepys, S.G. Thompson, R. Collins, et al., C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis, Lancet 375 (2010) 132–140.

[6] O. Yousuf, B.D. Mohanty, S.S. Martin, P.H. Joshi, M.J. Blaha, K. Nasir, et al., High-sensitivity C-reactive protein and cardiovascular disease: a resolute belief or an elusive link? J. Am. Coll. Cardiol. 62 (2013) 397–408.

[7] S. Devanarayanan, H. Nandeesha, S. Kattimani, S. Sarkar, J. Jose, Elevated copper, hs C-reactive protein and dyslipidemia in drug free schizophrenia: relation with psychopathology score, Asian J. Psychiatr. 24 (2016) 99–102.

[8] A.A. Saad, Y.A. Doka, S.M. Osman, M. Magzoub, N.I. Ali, I. Adam, Zinc, copper and C-reactive protein in children with severe Plasmodium falciparum malaria in an area of unstable malaria transmission in eastern Sudan, J. Trop. Pediatr. 59 (2013) 150–153.

[9] M. Wacewicz, K. Socha, J. Soroczynska, M. Niczyporuk, P. Aleksiejczuk, J. Ostrowska, et al., Concentration of selenium, zinc, copper, Cu/Zn ratio, total antioxidant status and c-reactive protein in the serum of patients with psoriasis treated by narrow-band ultraviolet B phototherapy: a case-control study, J. Trace Elem. Med. Biol. 44 (2017) 109–114.

[10] H. Noh, H.Y. Paik, J. Kim, J. Chung, The alteration of zinc transporter gene expression is associated with inflammatory markers in obese women, Biol. Trace Elem. Res. 158 (2014) 1–8.

[11] K.C. Poudel, E.R. Bertone-Johnson, K. Poudel-Tandukar, Serum zinc concentration and C-reactive protein in individuals with human immunodeficiency virus infection: the positive living with HIV (POLH) study, Biol. Trace Elem. Res. 171 (2016) 63–70.

[12] R.A. Ghashut, D.C. McMillan, J. Kinsella, A.T. Vasilaki, D. Talwar, A. Duncan, The effect of the systemic inflammatory response on plasma zinc and selenium adjusted for albumin, Clin. Nutr. 35 (2016) 381–387.

[13] P. Xun, K. Liu, J.S. Morris, M.L. Daviglus, J. Stevens, D.R. Jacobs Jr.et al., Associations of toenail selenium levels with inflammatory biomarkers of fibrinogen, high-sensitivity c-reactive protein, and interleukin-6: the CARDIA trace element study, Am. J. Epidemiol. 171 (2010) 793–800.

[14] M. Maktabi, M. Jamilian, Z. Asemi, Magnesium-zinc-calcium-vitamin D co-supplementation improves hormonal profiles, biomarkers of inflammation and oxidative stress in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial, Biol. Trace Elem. Res. 182 (2018) 21–28.

[15] L.E. Simental-Mendia, A. Sahebkar, M. Rodriguez-Moran, G. Zambrano-Galvan, F. Guerrero-romero, Effect of magnesium supplementation on plasma C-reactive protein concentrations: a systematic review and meta-analysis of randomized controlled trials, Curr. Pharmaceut. Des. 23 (2017) 4678–4686.

[16] A.Z. Pollack, S.L. Mumford, L. Sjaarda, N.J. Perkins, F. Malik, J. Wactawski-Wende, et al., Blood lead, cadmium and mercury in relation to homocysteine and C-reactive protein in women of reproductive age: a panel study, Environ. Health 16 (2017) 84.

[17] V.Q. Bui, A.D. Stein, A.M. DiGirolamo, U. Ramakrishnan, R.C. Flores-Ayala, M. Ramirez-Zea, et al., Associations between serum C-reactive protein and serum zinc, ferritin, and copper in Guatemalan school children, Biol. Trace Elem. Res. 148 (2012) 154–160.

[18] M. Kanai, M. Akiyama, A. Takahashi, N. Matoba, Y. Momozawa, M. Ikeda, N. Iwata, et al., Genetic analysis of quantitative traits in the Japanese population links cell types to complex human diseases, Nat. Genet. 50 (2018) 390–400.

[19] K.N. Kim, M.R. Lee, Y.H. Lim, Y.C. Hong, Blood lead levels, iron metabolism gene polymorphisms and homocysteine: a gene-environment interaction study, Occup. Environ. Med. 74 (2017) 899–904.

[20] R. Uher, Gene-environment interactions in common mental disorders: an update and strategy for a genome-wide search, Soc. Psychiatry Psychiatr. Epidemiol. 49 (2014) 3–14.

[21] E.K. Speliotes, C.J. Willer, S.I. Berndt, K.L. Monda, G. Thorleifsson, A.U. Jackson, et al., Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index, Nat. Genet. 42 (2010) 937–948.

[22] F. Wang, J. Zhu, P. Yao, X. Li, M. He, Y. Liu, et al., Cohort profile: the Dongfeng-Tongji cohort study of retired workers, Int. J. Epidemiol. 42 (2013) 731–740.

[23] Y. Yuan, Y. Xiao, W. Feng, Y. Liu, Y. Yu, L. Zhou, et al., Plasma metal concentrations and incident coronary heart disease in Chinese adults: the Dongfeng-Tongji cohort, Environ. Health Perspect. 125 (2017) 107007.

[24] Y. Yuan, Y. Xiao, Y. Yu, Y. Liu, W. Feng, G. Qiu, et al., Associations of multiple plasma metals with incident type 2 diabetes in Chinese adults: the Dongfeng-Tongji cohort, Environ. Pollut. 237 (2018) 917–925.

[25] D.J. Paustenbach, J.M. Panko, M.M. Fredrick, B.L. Finley, D.M. Proctor, Urinary chromium as a biological marker of environmental exposure: what are the limitations? Regul. Toxicol. Pharmacol. 26 (1997) S23–S34.

[26] H.J. Wiegand, H. Ottenwalder, H.M. Bolt, Recent advances in biological monitoring of hexavalent chromium compounds, Sci. Total Environ. 71 (1988) 309–315.

[27] Y.C. Ma, L. Zuo, J.H. Chen, Q. Luo, X.Q. Yu, Y. Li, et al., Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease, J. Am. Soc. Nephrol. 17 (2006) 2937–2944.

[28] M. He, C. Wu, J. Xu, H. Guo, H. Yang, X. Zhang, et al., A genome wide association study of genetic loci that influence tumour biomarkers cancer antigen 19-9, carcinoembryonic antigen and alpha fetoprotein and their associations with cancer risk, Gut 63 (2014) 143–151.

[29] M.A. He, M. Xu, B. Zhang, J. Liang, P. Chen, J.Y. Lee, et al., Meta-analysis of genome-wide association studies of adult height in East Asians identifies 17 novel loci, Hum. Mol. Genet. 24 (2015) 1791–1800.

[30] N. Pike, Using false discovery rates for multiple comparisons in ecology and evolution, Methods Ecol. Evol. 2 (2011) 278–282.

[31] M. Wisniewska, M. Cremer, L. Wiehe, N.P. Becker, E. Rijntjes, J. Martitz, et al., Copper to zinc ratio as disease biomarker in neonates with early-onset congenital infections, Nutrients 9 (2017).

[32] A.L. Price, N.J. Patterson, R.M. Plenge, M.E. Weinblatt, N.A. Shadick, D. Reich, Principal components analysis corrects for stratification in genome-wide association studies, Nat. Genet. 38 (2006) 904–909.

[33] F. Faul, E. Erdfelder, A. Buchner, A.-G. Lang, Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses, Behav. Res. Methods 41 (2009) 1149–1160.

[34] M. Michalska-Mosiej, K. Socha, J. Soroczynska, E. Karpinska, B. Lazarczyk, M.H. Borawska, Selenium, zinc, copper, and total antioxidant status in the serum of patients with chronic tonsillitis, Biol. Trace Elem. Res. 173 (2016) 30–34.

[35] E. Mocchegiani, L. Costarelli, R. Giacconi, M. Malavolta, A. Basso, F. Piacenza, et al., Micronutrient-gene interactions related to inflammatory/immune response and antioxidant activity in ageing and inflammation. A systematic review, Mech. Ageing Dev. 136–137 (2014) 29–49.

[36] P. Bonaventura, G. Benedetti, F. Albarede, P. Miossec, Zinc and its role in immunity and inflammation, Autoimmun. Rev. 14 (2015) 277–285.

[37] B.R. Stern, Essentiality and toxicity in copper health risk assessment: overview, update and regulatory considerations, J. Toxicol. Environ. Health 73 (2010) 114–127.

[38] M. Tanaka, G.K. Mokhtari, R.D. Terry, L.B. Balsam, K.H. Lee, T. Kofidis, et al., Overexpression of human copper/zinc superoxide dismutase (SOD1) suppresses ischemia-reperfusion injury and subsequent development of graft coronary artery disease in murine cardiac grafts, Circulation 110 (2004) II200–206.

[39] A.S. Prasad, Zinc in human health: effect of zinc on immune cells, Mol. Med. 14 (2008) 353–357.

[40] M.P. Rayman, Selenium and human health, Lancet 379 (2012) 1256–1268.

[41] E.S. Ford, S. Liu, D.M. Mannino, W.H. Giles, S.J. Smith, C-reactive protein concentration and concentrations of blood vitamins, carotenoids, and selenium among United States adults, Eur. J. Clin. Nutr. 57 (2003) 1157–1163.

[42] A.C. Scheurig, B. Thorand, B. Fischer, M. Heier, W. Koenig, Association between the intake of vitamins and trace elements from supplements and C-reactive protein: results of the MONICA/KORA Augsburg study, Eur. J. Clin. Nutr. 62 (2008) 127–137.

[43] A. Farrokhian, F. Bahmani, M. Taghizadeh, S.M. Mirhashemi, M.H. Aarabi, F. Raygan, et al., Selenium supplementation affects insulin resistance and serum hs-CRP in patients with type 2 diabetes and coronary heart disease, Horm. Metab. Res. 48 (2016) 263–268.

[44] R. Freitas, R.J.N. Nogueira, S.M.F. Cozzolino, A.C.J. Vasques, G. Hessel, Influence of selenium supplementation on patients with inflammation: a pilot double blind randomized study, Nutrition 41 (2017) 32–36.

[45] E.E. Battin, M.T. Zimmerman, R.R. Ramoutar, C.E. Quarles, J.L. Brumaghim, Preventing metal-mediated oxidative DNA damage with selenium compounds, Metallomics 3 (2011) 503–512.

[46] P.M. Ridker, A test in context: high-sensitivity C-reactive protein, J. Am. Coll. Cardiol. 67 (2016) 712–723.

[47] T.C. Pereira, M.M. Campos, M.R. Bogo, Copper toxicology, oxidative stress and inflammation using zebrafish as experimental model, J. Appl. Toxicol. 36 (2016) 876–885.

[48] X. Sun, J. Li, H. Zhao, Y. Wang, J. Liu, Y. Shao, et al., Synergistic effect of copper and arsenic upon oxidative stress, inflammation and autophagy alterations in brain tissues of Gallus gallus, J. Inorg. Biochem. 178 (2018) 54–62.

[49] H. Speisky, M. Gomez, F. Burgos-Bravo, C. Lopez-Alarcon, C. Jullian, C. Olea-Azar, et al., Generation of superoxide radicals by copper-glutathione complexes: redox-consequences associated with their interaction with reduced glutathione, Bioorg. Med. Chem. 17 (2009) 1803–1810.

[50] Q. Qi, A.Y. Chu, J.H. Kang, M.K. Jensen, G.C. Curhan, L.R. Pasquale, et al., Sugar-sweetened beverages and genetic risk of obesity, N. Engl. J. Med. 367 (2012) 1387–1396.

[51] S. Li, J.H. Zhao, J. Luan, U. Ekelund, R.N. Luben, K.T. Khaw, et al., Physical activity attenuates the genetic predisposition to obesity in 20,000 men and women from EPIC-Norfolk prospective population study, PLoS Med. 7 (2010) e1000332.

[52] T. Huang, T. Wang, Y. Heianza, Y. Zheng, D. Sun, J.H. Kang, et al., Habitual consumption of long-chain n-3 PUFAs and fish attenuates genetically associated long-term weight gain, Am. J. Clin. Nutr. 109 (2019) 665–673.

[53] J. Belsky, M. Pluess, Beyond diathesis stress: differential susceptibility to environmental influences, Psychol. Bull. 135 (2009) 885–908.

[54] J. Belsky, C. Jonassaint, M. Pluess, M. Stanton, B. Brummett, R. Williams, Vulnerability genes or plasticity genes? Mol. Psychiatry 14 (2009) 746–754.

[55] Y. Okada, A. Takahashi, H. Ohmiya, N. Kumasaka, Y. Kamatani, N. Hosono, et al., Genome-wide association study for C-reactive protein levels identified pleiotropic associations in the IL6 locus, Hum. Mol. Genet. 20 (2011) 1224–1231.

[56] P.M. Ridker, G. Pare, A. Parker, R.Y. Zee, J.S. Danik, J.E. Buring, et al., Loci related to metabolic-syndrome pathways including LEPR,HNF1A, IL6R, and GCKR

associate with plasma C-reactive protein: the Women's Genome Health Study, Am. J. Hum. Genet. 82 (2008) 1185–1192.
[57] K.L. Rasmussen, Plasma levels of apolipoprotein E, APOE genotype and risk of dementia and ischemic heart disease: a review, Atherosclerosis 255 (2016) 145–155.
[58] D.I. Chasman, P. Kozlowski, R.Y. Zee, D.J. Kwiatkowski, P.M. Ridker, Qualitative and quantitative effects of APOE genetic variation on plasma C-reactive protein, LDL-cholesterol, and apoE protein, Genes Immun. 7 (2006) 211–219.
[59] H. Xu, D.I. Finkelstein, P.A. Adlard, Interactions of metals and apolipoprotein E in alzheimer's disease, Front. Aging Neurosci. 6 (2014) 121.
[60] A.A. Arguinano, E. Naderi, N.C. Ndiaye, M. Stathopoulou, S. Dadé, B. Alizadeh, et al., IL6R haplotype rs4845625*T/rs4537545*C is a risk factor for simultaneously high CRP, LDL and ApoB levels, Genes Immun. 18 (2017) 163–169.
[61] R. Kerr, D. Stirling, C.A. Ludlam, Interleukin 6 and haemostasis, Br. J. Haematol. 115 (2001) 3–12.
[62] K.G. Lockwood, A.L. Marsland, S. Cohen, P.J. Gianaros, Sex differences in the association between stressor-evoked interleukin-6 reactivity and C-reactive protein, Brain Behav. Immun. 58 (2016) 173–180.
[63] V. Ansteinsson, M. Refsnes, T. Skomedal, J.B. Osnes, I. Schiander, M. Låg, Zinc- and copper-induced interleukin-6 release in primary cell cultures from rat heart, Cardiovasc. Toxicol. 9 (2009) 86–94.
[64] A.H. Shadyab, R. Terkeltaub, C. Kooperberg, A. Reiner, C.B. Eaton, R.D. Jackson, et al., Prospective associations of C-reactive protein (CRP) levels and CRP genetic risk scores with risk of total knee and hip replacement for osteoarthritis in a diverse cohort, Osteoarthr. Cartil. 26 (2018) 1038–1044.