The association between blood copper concentration and biomarkers related to cardiovascular disease risk – analysis of 206 individuals in the Northern Finland Birth Cohort 1966

Saranya Palaniswamy[1,2], Terhi Piltonen[3], Markku Koiranen[1], Darja Mazej[4], Marjo-Riitta Järvelin[1,2,5,6], Khaled Abass[7,8], Arja Rautio[7*], Sylvain Sebert[1,2,9*]

1. Center For Life Course Health Research, Faculty of Medicine, University of Oulu, FI-90014, Oulu, Finland.
2. Biocenter Oulu, University of Oulu, FI-90014, Oulu, Finland.
3. Department of Obstetrics and Gynecology, Oulu University Hospital, University of Oulu and PEDEGO Research Unit, P.O. Box 23, FI-90029 Oulu University Hospital, Oulu, Finland; Medical Research Center Oulu, Oulu University Hospital and University of Oulu, P.O. Box 8000, FI-90014 University of Oulu, Oulu, Finland.
4. Department Environmental Sciences, Jozef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia.
5. Department of Epidemiology and Biostatistics, MRC-PHE Centre for Environment and Health, School of Public Health, Imperial College London, London SW7 2AZ, United Kingdom.
6. Oulu University Hospital, Unit of Primary Care, FI-90014, Oulu, Finland.
7. Arctic Health, Faculty of Medicine, University of Oulu, FI-90014 Oulu, Finland.
8. Department of Pesticides, Menoufia University, P.O. Box 32511, Egypt.
9. Department of Genomics of Complex Diseases, School of Public Health, Imperial College London, London SW7 2AZ, United Kingdom.

***Corresponding authors**
Docent. Sylvain Sebert and Professor. Arja Rautio,
Center For Life Course Health Research,
Arctic Health, Faculty of Medicine, Aapistie 5B, 90014,
University of Oulu, Oulu, Finland.
Mail: sylvain.sebert@oulu.fi; arja.rautio@oulu.fi;
Tel: *+358 503 44 08 42*
Fax: +358 8 537 5661

Correspondence (manuscript): Dr. Saranya Palaniswamy, Center For Life Course Health Research, Faculty of Medicine, Aapistie 5B, 90014, University of Oulu, Oulu, Finland.
Mail: saranya.palaniswamy@oulu.fi
Tel: +358 417541076

Short title: Copper and biomarkers of cardiovascular disease risk

Abstract

Background: Copper is an abundant trace element in humans where alterations in the circulating concentration could inform on chronic disease aetiology. To date, data are lacking to study how copper may associate with cardiovascular disease (CVD) risk factors in young and healthy population. Molecular evidence suggests an important role of copper in liver metabolism, an essential organ in maintaining cardiovascular health and inflammation, therefore supporting copper as an associated biomarker of the risk.

Objective: We performed a cross-sectional analysis to examine the possible associations between blood copper levels and risk factors for CVD and pre-inflammatory process.

Design: The data has been collected from a sub-sample set of the Northern Finland Birth Cohort 1966 (NFBC1966) at 31 years.

Participants: The study included 206 individuals, 116 men and 90 women. To reduce environmental individual variations affecting both copper and the metabolic profile in the study sample, the participants were selected as: i) being born in Finnish Lapland and ii) living in their birth place for the last five years preceding blood sampling.

Main outcome measures: Fasting blood copper concentration was measured by inductively coupled plasma mass spectrometer. The CVD risk factors included 6 metabolic clusters (30 cardiovascular and pro-inflammatory factors) assessed by nuclear magnetic resonance. Multivariate linear regression analysis was performed to test the linear association between blood copper and 6 metabolic clusters for CVD risk. Associations were assessed under correction for multiple testing.

Results: Copper (Cu) levels were comparable in men and women, with no difference between sexes (*p-value* <0.60). In multiple regression models, sex adjusted, copper was associated with 9 metabolites from 4 metabolic clusters. After adjustment with BMI, copper was associated with 4 metabolites from 3 metabolic clusters: glutamine, beta-hydroxybutyrate, alpha-1-acid glycoprotein (AGP) and high-sensitive C-reactive protein (hs-CRP). After correction for multiple testing, Cu was found positively associated with only 2 biomarkers of inflammation including AGP [p=0.04] and hs-CRP [p=0.0001].

Conclusions: Considering the strength and limitation of the study design, the present study does not support evidence for an independent role of copper on biomarkers for CVD risk. Nevertheless, we are reporting a robust association of copper with the inflammatory load that is important to consider in light with the

inflammatory component of chronic health. In addition, the association of copper with metabolites may be attributable to BMI or environmental factors associated to it, and warrants further research in large population samples.

Keywords: Copper, metabolic profile, young adult, cardiovascular disease risk, metabolomics, inflammatory biomarkers

Introduction

Cardiovascular disorders and diseases are a major cause of death where scientific efforts are needed to identify early risk factors and potential preventive biomarkers in non-affected populations. After Iron and Zinc, Copper (Cu) is the third most important trace element circulating in the body [1,2] originating principally from dietary sources *i.e.* food and water [2]. Cu^{++} can be extremely toxic due to its oxidoreductase function [3,4] and is transported in the circulation by the carrier-protein ceruloplasmin [5]. There is contrasting evidence for the influence of Cu on pro- or anti-oxidative pathways and subsequent risk of atherosclerosis in humans [6]. To-date Cu plays a role in nineteen enzymatic reactions involved in oxidative stress and metabolic homeostasis [4] including the Cu/Zn dependent superoxide dismutase (SOD), the cytochrome *c* oxidase and the ceruloplasmin [4]. In addition, there is no clinical threshold defining a healthy range of fasting blood copper. While modulation of dietary intake of copper could be implemented as a strategy to influence cardiometabolic health [7,8], fundamental questions remains to be solved before clear recommendation can be announced. A wider knowledge of the metabolic pathways linking copper to altered cardiovascular health can be needed. Following the advent of wider metabolomic profiling [9] we are gaining a dimension in the molecular pathways involved aside from the classical markers of cardiovascular disease especially LDL-C, triglycerides and HDL-C [10]. This includes variations in lipoprotein structure and composition, some amino-acids, ketone bodies and pro-inflammatory factors [11]. To date 30 metabolites are suggested to influence cardiovascular health and might be influenced by copper concentration of copper metabolism related pathways [10].

In line with the biological functions of copper and the current bodies of evidence, the present study aimed at testing the hypothesis, in a non-affected sample of young and healthy adults at 31 years, that fasting blood copper is associated with CVD risk biomarkers as defined from previous large scale metabolomic study [10]. Identifying potential new biomarkers such as copper might help detecting very early disease state with the aim

100 to also inform on the molecular pathway involved. The objectives of the present study were therefore to
101 characterise blood concentration in a young and healthy non-affected sample with cardiovascular disease and
102 test the linear association of copper with known risk factors established from the study of the metabolome and
103 inflammatory load related to the etiology of chronic cardio-metabolic diseases.
104
105 **Materials and Methods**
106 ***Study design and material***
107 The study was based on the 31-year follow-up of the Northern Finland Birth Cohort (NFBC1966) [12]. Briefly
108 the NFBC1966 is a prospective birth cohort of 12,058 (96% of total population) live-born individuals in the
109 province of Oulu (65 degree North) and Lapland in year 1966 [12,13]. The inclusion criteria for the present
110 study were i) being born in Finnish Lapland and ii) living in their birthplace for the last five years preceding
111 fasting blood sampling and iii) complete case analysis. Due to economic constraint, copper was measured only
112 in 126 males and 122 females. The selection criteria aimed at reducing environmental variations known to
113 affect both copper and the metabolic profile. We excluded pregnant women (N=6), oral contraceptive users
114 (N=16), and hs-CRP>10 mg/L (N=6) (acute inflammation) as these factors are associated with variation in the
115 metabolic profile [14]. The complete case analysis data consisted of N=206 subjects (N=20 subjects missing
116 with one or more of the covariates and metabolomics data examined). The anthropometric, socio-demographic
117 and lifestyle characteristics of the population are described in Table 1 (see [15] for detailed protocols). All
118 participants gave written informed consents and the Ethics Committee of Northern Ostrobothnia hospital
119 district and the University of Oulu (Finland) approved the study.
120 This study was performed on a healthy adult population at 31 years with overnight fasted blood samples (~7-
121 8 hours). The overnight fasted state was chosen to reduce inter-individual variability on the potential outcomes
122 also limiting the influence on the oxidative phosphorylation/fatty-acid oxidation with the synthesis of ketone
123 body or any circulating metabolite examined in the study. The alcohol consumption of the participants
124 described in the **Table 1** was obtained from the postal questionnaire data on the frequency of consumption of
125 various alcoholic beverages during the previous six months at 31 years [15]. Participant's invited for the
126 clinical examination was requested to abstain from alcohol consumption before blood sampling. There was no
127 difference in copper concentrations with the categories of alcohol consumption examined in our study (*p*-

value=0.46). In addition, participants included in the study have not reported history of liver disease or any other metabolic diseases.

Biochemical analyses

The present measure of Cu concentration was performed in total blood as plasma or serum Cu concentrations are considered less precise indicators for the total body copper status [16], where previous report failed to observe changes in plasma Cu or serum Cu concentrations with Cu supplementation or Cu intake [16].

12-hour overnight fasted blood samples were collected in metal-free tubes following an immediate freezing in -80°C for further analyses. An aliquot of 0.3 mL of blood sample was diluted ten times with an alkaline solution containing Triton X-100 and ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA) [17]. For calibration the standard addition procedure was performed. Measurement of copper in prepared solutions were made by an Octapole Reaction System (ORS) Inductively Coupled Plasma Mass Spectrometer (ICP-MS, 7500ce, Agilent) equipped with an ASX-510 Autosampler (Cetac). Instrumental conditions: Babington nebulizer, Scott-type spray chamber, spray chamber temperature 5 °C, plasma gas flow rate 15 L/min, carrier gas flow rate 0.8 L/min, make-up gas flow rate 0.1 L/min, RF power 1500 W, reaction cell gas helium 4,3 mL/min, isotope monitored ^{65}Cu. Analytical precision was 5%. The accuracy of results was checked by analysing Seronorm Trace Elements Whole Blood L-1 (SERO AS, Norway) as a reference material. The measured values were in good agreement with reference values.

Inflammatory biomarkers

Serum high-sensitivity C-reactive protein (hs-CRP, mg/L) concentration was measured by immunoenzymometric assay (Medix Biochemica, Espoo, Finland). The intra- and inter-assay coefficients of variation (CV) were 4.2% and 5.2%.

Active plasminogen activator inhibitor-1 (active PAI-1, ng/ml) concentration was measured by multiplex array method using non-magnetic human CVD/cytokine panel (SPR349, Merck Millipore, USA) according to manufacturer's instructions. The intra- and inter-assay CV were 6.8% and 18.6%.

Alpha-1-acid glycoprotein (AGP) and other metabolite measurements: Alpha-1-acid glycoprotein (mmol/L) was extracted along with a group of 123 metabolites in NFBC1966. The quantification of the metabolomics biomarkers including AGP was performed by Proton Nuclear Magnetic Resonance (NMR) as described in

Würtz *et al.,* (2015) [10]. The metabolomics platform is based on three molecular windows (lipoprotein, low-molecular-weight metabolite data and lipid) for each sample [10].

Grouping of the 6 clusters of biomarkers: The circulating metabolites were reported as strong predictors of CVD risk as the conventional lipid risk factors, and were markers of CVD onset during more than a decade follow-up [10]. In addition, the biomarker associations were replicated in independent cohorts with varying baseline characteristics including age and ethnicity, and were consistent across different metabolite profiling platforms [10]. Therefore, to reduce the number of hypotheses tested and to account for the small sample size of the current study we chose to select only the sentinel metabolites from the six metabolic clusters available by the NMR metabolic methods (see supplementary figure from the article Würtz *et al.,* (2015, figure 2) [10]). In total 30 metabolites showing significant association with cardiovascular events were selected [10]. The metabolites and inflammatory biomarkers were categorised under 6 clusters as reported in Würtz *et al.,* (2015, supplementary figure 2 from the article [10]). Cluster 1, amino acids including branched-chain amino acids, aromatic amino acids (alanine, glutamine, isoleucine, phenyl alanine, tyrosine), cluster 2, glycolysis related metabolites including ketone bodies (glucose, lactate, pyruvate, glycerol, beta-hydroxy butyrate), cluster 3, fatty acids (total fatty acids, monounsaturated fatty acids (MUFA), saturated fatty acids(SFA)), cluster 4, apolipoproteins (apolipoprotein B, apolipoprotein A1), cluster 5, lipids including total lipid concentration in lipoprotein subclasses (triglycerides, extremely large very large density lipoprotein (VLDL), very large VLDL, large VLDL, medium VLDL, small VLDL, very small VLDL, intermediate-density lipoprotein (IDL), large low density lipoprotein (LDL), medium LDL, small LDL, large HDL, medium high density lipoprotein (HDL), small HDL) and cluster 6, inflammatory biomarkers (hs-CRP, alpha-1-acid glycoprotein (AGP), activePAI-1).

Statistical analysis

Statistical analyses were conducted using SAS software version 9.4 (SAS Institute Inc., Cary, USA). Due to incomplete information on one or more of the metabolites with copper concentration, 20 subjects were excluded from the study. The difference between complete and incomplete data set was examined by Kruskal-Wallis test and Pearson's Chi-square test. Normality for all variables was assessed and natural log-transformation was done accordingly. The comparisons of Cu concentration between groups were analysed

using Student's *t*-test for continuous variables and Chi-square for categorical. A multiple linear regression analyses using standardised score (z-scores; mean=0; SD=1) was then performed to study the association between copper concentration and the metabolites (model 1, unadjusted). The linear model was adjusted for sex (model 2), and further adjusted for BMI (model 3). *P*-values were adjusted for multiple testing using the Benjamin and Hochberg (1995) false discovery rate (FDR) approach separately for each cluster (n=6) [18]. The statistical tests were two-sided and significance was considered at $p<0.05$.

Results

Characteristics of participants. General characteristics of the study participants are summarised in **Table 1**. The data analysis was performed on N=206 individuals (116 men and 90 women). We found no statistically significant differences between men and women for anthropometric, lifestyle and socio-demographic characteristics (*p*-value>0.05). In addition, we found no difference between complete and incomplete data analysis (data not shown).

Copper and CVD risk markers. Figure 1 shows results from the multivariable regression analyses of the association between copper and six metabolic clusters and inflammatory biomarkers examined in the study. In model 1, unadjusted, copper was positively associated with metabolites phenylalanine, very small VLDL, IDL, alpha-1-acid glycoprotein and hs-CRP (*p*-value<0.05). In addition, in model 1, copper was negatively associated with metabolite glutamine (*p*-value<0.05). In model 2, adjusted for sex, the strength of association was almost identical between Cu and the above mentioned metabolites. In addition, the strength of the association between Cu and few other additional metabolites attained significant effect after adjustment for sex including, apolipoprotein A1, large LDL, and large HDL. In model 3, after adjustment for BMI, the association between Cu and all the other metabolites were attenuated, except for glutamine, beta-hydroxy butyrate, alpha-1-acid glycoprotein and hs-CRP. After correction for multiple testing using FDR-approach, Cu was found to be only positively associated with inflammatory biomarkers, alpha-1-acid glycoprotein (β=0.14, *P*-value correction=0.04) and hs-CRP (β=0.28, *P*-value correction=0.0001). Copper was not associated with PAI-1 in any of the three models examined in our study (*P*-value>0.05).

Discussion

212 Copper is both a pro-oxidant and antioxidant [6]. Copper plays a role in nineteen enzymatic reactions involved
213 in oxidative stress and metabolic homeostasis [4] including the Cu/Zn dependent superoxide dismutase (SOD),
214 the cytochrome *c* oxidase and the ceruloplasmin [4]. On the other hand, copper ions participate in radical
215 reactions such as conversion of superoxide to hydrogen peroxide and hydroxyl radicals, and catalyse the
216 oxidative modification of LDL in vitro [19,20]. High serum Cu has been reported as an independent risk factor
217 for CVD disease in multiple epidemiological studies [6,21,22] which show the duplicitous nature of copper.
218 However, these results should be interpreted with caution as the studies were performed in wider age groups,
219 no adjustment for BMI/inflammatory biomarkers, small population size or in diseased populations [6,21,22].

220 ***Copper and circulating metabolites – BMI as a main effect modulator?***

221 From the sex-adjusted model, we observed copper to be associated with 9 metabolites (phenylalanine, very
222 small VLDL, IDL, apolipoprotein A1, large LDL, large HDL, glutamine, alpha-1-acid glycoprotein and hs-
223 CRP) in the multiple regression analyses. However, adjustment for BMI in model 3 strongly attenuated these
224 associations suggesting an important modification either by BMI or the environmental factors associated to it.
225 The environmental factors include an unhealthy diet, sedentary behaviour, and physical inactivity. Adjustment
226 of these factors in the analyses would have provided information on whether the attenuation of the association
227 between copper and metabolites were entirely based on BMI or confounded by the environmental factors
228 associated to it. Only one previous investigation on 231 participants has reported significant association
229 between serum copper and classical markers (total cholesterol, LDL-cholesterol) of CVD risk adjusting for
230 BMI [23]. Few studies has reported an increase in serum copper concentrations in overweight and obese
231 participants compared with normal weight participants [24,25]. Another study (N=189) has reported an
232 increase in copper concentrations with categories of BMI [26]. However, no difference were observed between
233 total blood copper concentration and BMI categories in our study (*p*-value=0.07, **Table 1**). Only limited
234 information is available in the literature on the influence of BMI on copper concentration in healthy young
235 population. These results may show that increase in Cu concentrations and link to CVD risk may be influenced
236 by lifestyle factors and future studies are warranted to test this association.

237 ***Copper and inflammation*** (Figure 2)

238 Copper plays an essential role in regulating reactive oxygen and nitrogen species (ROS/RNS) concentration
239 and, variations in Cu metabolism could modulate various inflammatory pathways [27]. Copper was found to

be positively associated with alpha-1-acid glycoprotein and hs-CRP in our study. In fact, the present data supports a role for Cu in modulating systemic inflammation with a robust association between Cu and inflammatory biomarkers. Our results confirm the previously reported association between copper and inflammation [23,26], thus adding to the body of evidence linking copper with the alteration of inflammatory biomarkers.

Alpha-1-acid glycoprotein and CRP are acute-phase inflammatory biomarkers, and may play a critical role in the aetiology of low-grade inflammation status as they are frequently reported in relation to obesity and CVD events [28]. In our cross-sectional study we sought to identify the elements that promote systemic inflammation in relation to copper which may offer insights into the development of CVD, thus suggesting targets for CVD prevention. However, the association between copper and inflammatory biomarkers, should be interpreted with caution in the light of interplay between the factors copper, oxidative stress and inflammation **(Figure 2)**. Under inflammatory conditions, serum copper levels are increased and trigger oxidative stress responses that activate inflammatory responses [27]. Interestingly, copper dyshomeostasis, oxidative stress and inflammation are commonly present in several chronic diseases including obesity, neurodegenerative diseases and CVD **(Figure 2)**. There are still controversies on the causal and consequential interconnections of copper, oxidative stress and inflammation which warrants for further epidemiological studies to carefully interpret the above mentioned associations **(Figure 2)**.

Copper and plasminogen activator inhibitor-1

Among the list of other risk markers for cardiovascular events, it has been proposed that impairment of the fibrinolytic system detected in plasma, due to increased plasminogen activator inhibitor-1 (PAI-1) concentration, could predict the complications of CVD [29]. PAI-1 is responsible for the specific and rapid inhibition of fibrinolytic proteases, thus maintaining proper homeostasis and stringent control with the fibrinolysis system, thrombosis and vascular remodelling [30]. However, active PAI-1 is inherently unstable and readily undergoes a significant structural rearrangement and converts to an inactive, latent form that can no longer inhibit fibrinolytic proteases [31]. Metal ion ligands such as copper are reported to modulate the stability of PAI-1 [31]. Copper binds to PAI-1 with high affinity, resulting in significant acceleration in the

rate of latency conversion [31]. Few studies has reported an association of copper ions in interfering with the stability of PAI-1 concentration in the circulation [31,32].

Previous investigation on healthy women (N=16) has reported a significant decrease in PAI-1 concentration with Cu supplementation [16]. This is in contrast from our report where Cu concentration was not associated with PAI-1 in our study. This may be because of inclusion of both sexes and higher sample size in our study when compared to the previous investigation in women only [16]. In addition, previous investigation has not accounted for rigorous statistical procedures including multiple testing and Cu in our study was measured from the blood samples in normal physiological state rather than from supplementation.

Strengths and limitations

The present analysis supported evidence for an association of Cu and an atherogenic metabolic profile that might be influenced by sex and body mass index in humans. Unfortunately, the accurate assessment of Cu concentration requires expensive ICP-MS analysis on blood samples collected on metal-free tubing, which will limit the opportunity for a large-scale data analysis. The strength of the present approach lies in the fact that the exploration is performed in a representative sample of young adult subjects with equal number of males and females. Moreover, the study sample was selected as regards geographical area for living in order to limit dietary and environmental exposure. In addition, the total blood copper concentration measured in the present study was consistent with previously published values suggestive of a healthy physiological range in the NFBC1966 participants [33]. We were unable to quantify other indicators related to Cu status such as ceruloplasmin and the Cu-dependent enzymes (erythrocyte superoxide dismutase (SOD), cytochrome C oxidase activity) which could have provided additional information on the copper metabolism.

We have measured the circulating metabolites from serum which are considered to be more sensitive and reproducible in biomarker detection when compared to plasma samples [34]. Metabolomics gives an instantaneous snapshot of the metabolism and cannot be translated into a dynamic map of metabolite traffic on biochemical routes. However, we should be aware that we measured serum concentrations which may not necessarily reflect intracellular processes. As extensive metabolomics data are increasingly available, we expect in future comprehensive metabolic profiles from large epidemiologic studies which will elucidate molecular mechanism and pathways in relation to copper and CVD risk.

We have examined a wide range of cardiovascular risk factors in relation to copper concentration. However, the cross sectional study design does not ensure causality. The possibility of uncontrolled or unknown confounders cannot be ruled out. Interestingly, one genome wide association study (GWAS) was performed for blood copper concentration [35]. The data on 2603 Australian adults reported two genome wide significant variants, rs1175550 and rs2769264 associated with blood copper. To ascertain potential causal association between blood copper and the metabolic profile observed in our present analysis, we search for association between the Cu SNPs from [35] on the summary statistics provided from the last GWAS on nuclear magnetic resonance metabolomics profile [36]. Unfortunately, both Cu-SNPs did not show association for any of the metabolites from a large GWAS catalogue, therefore suggesting a non-causal association. In addition, it is important to mention that the present study was performed using a limited study sample and further large sample population studies are needed to confirm an association between copper and biomarkers of cardiovascular disease risk. Finally, the study was performed on an ethnically homogeneous population of the Northern Finland which reduces the ability to generalise our results and warrants for multicentre studies to confirm if these results could be translated to the general population.

Conclusion

Our data together with others support an association between Cu and non-specific biomarkers of inflammation which may be related to ongoing proinflammatory process *in vivo* and warrants further research to understand the role of Cu in the aetiology of chronic diseases. The study warrants replication of the results in future large epidemiological investigations with different population characteristics which could help in early intervention and prevention of cardiovascular disease risk.

Competing Interests - None.

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Contributions: SP, AR and SS designed the analysis. SP conducted the analysis and wrote the manuscript with guidance from TP, AR, KA and SS. DM was responsible for blood metal concentration measurements in this analysis. All authors contributed intellectually to the manuscript and approved the final version.

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Figures and Tables

Figure 1 shows the association of copper concentration with metabolic clusters examined in the study. The * represents the metabolites that were significant after the BH-false discovery rate correction.

Figure 2 represents the relationship between copper and inflammation.

Legend for Figure 2: Copper and inflammation. Copper induces oxidative stress through imbalance in antioxidant system that increase reactive oxygen and nitrogen species (ROS, RNS), which might induce the activation of signaling pathways that upregulate proinflammatory cytokines leading to inflammation. However, inflammation is a hallmark of several chronic diseases including obesity, cardiovascular and neurodegenerative diseases. There are still controversies on causal and consequential interconnections between copper, oxidative stress and inflammation: those are mentioned throughout the text and represented by dotted lines. hs-CRP - high sensitivity C-reactive protein.

Table 1: Descriptive statistics and fasting blood copper concentration by anthropometric, lifestyle and socio-demographic factors.

Table 1: Descriptive statistics and fasting blood copper concentration by anthropometric, lifestyle and socio-demographic factors.

	Total (N=206 – 195)**	**[Cu] ng/mL Mean (SD)**	***P*-value***
Age (years)	31		
Men (n %)	116 (56.31)	835.66 (154.49)	
Women (n %)	90 (43.69)	883.52 (146.49)	0.60
BMI (kg/m^2) [Mean (SD)]	25.23 (4.34)		
Normal weight (n %)	110 (53.4)	835.63 (137.78)	
Overweight and Obese (n %)	96 (46.6)	880.57 (165.38)	0.07
Socioeconomic status (n %)[1]			
I + II (Professional)	24 (12.18)	894.50 (178.19)	
III (Skilled worker)	56 (28.43)	876.91 (123.76)	
IV (Unskilled worker)	66 (33.50)	822.35 (169.22)	0.16
V (Farmer)	9 (4.57)	886.22 (127.96)	
VI (Other)	42 (21.32)	876.86 (150.09)	
Diet score[2] (n %)			
0-1	55 (27.50)	879.42 (145.59)	
2-3	116 (58.00)	862.82 (156.39)	0.10
4-5	29 (14.50)	805.34 (151.61)	
Smoking status[3] (n %)			
No smoker	109 (55.61	835.59 (144.38)	
Smokers	87 (44.39)	888.56 (159.82)	0.32
Alcohol consumption (g/day)[4] (n %)			
Abstainer	14 (7.18)	900.29 (198.01)	
Low risk drinker	172 (88.21)	854.66 (149.28)	0.46
At-risk drinker	9 (4.62)	891.78 (148.97)	

Values are presented as mean (standard deviation) or number (%).
**P*-value was calculated using Student's *t*-test for continuous variables and ANOVA for categorical variables.
** N varies due to missing data for some of the variables

1. Data available on N=197 individuals; N=9 observations missing with socioeconomic position
2. Data available on N=200 individuals; N=6 observations missing with diet score
3. Data available on N=196 individuals; N=10 observations missing with smoking status
4. Data available on N=195 individuals; N=11 observations missing with alcohol consumption; Alcohol classification according to WHO sex-specific classification as abstainer, low risk drinker (≤20 g/day and ≤40 g/day for women and men, respectively) or at-risk drinker (>20g/day and >40g/day for women and men, respectively).

Figure 1 shows the association of copper concentration with metabolic clusters examined in the study. The * represents the metabolites that were significant after the BH-false discovery rate correction.

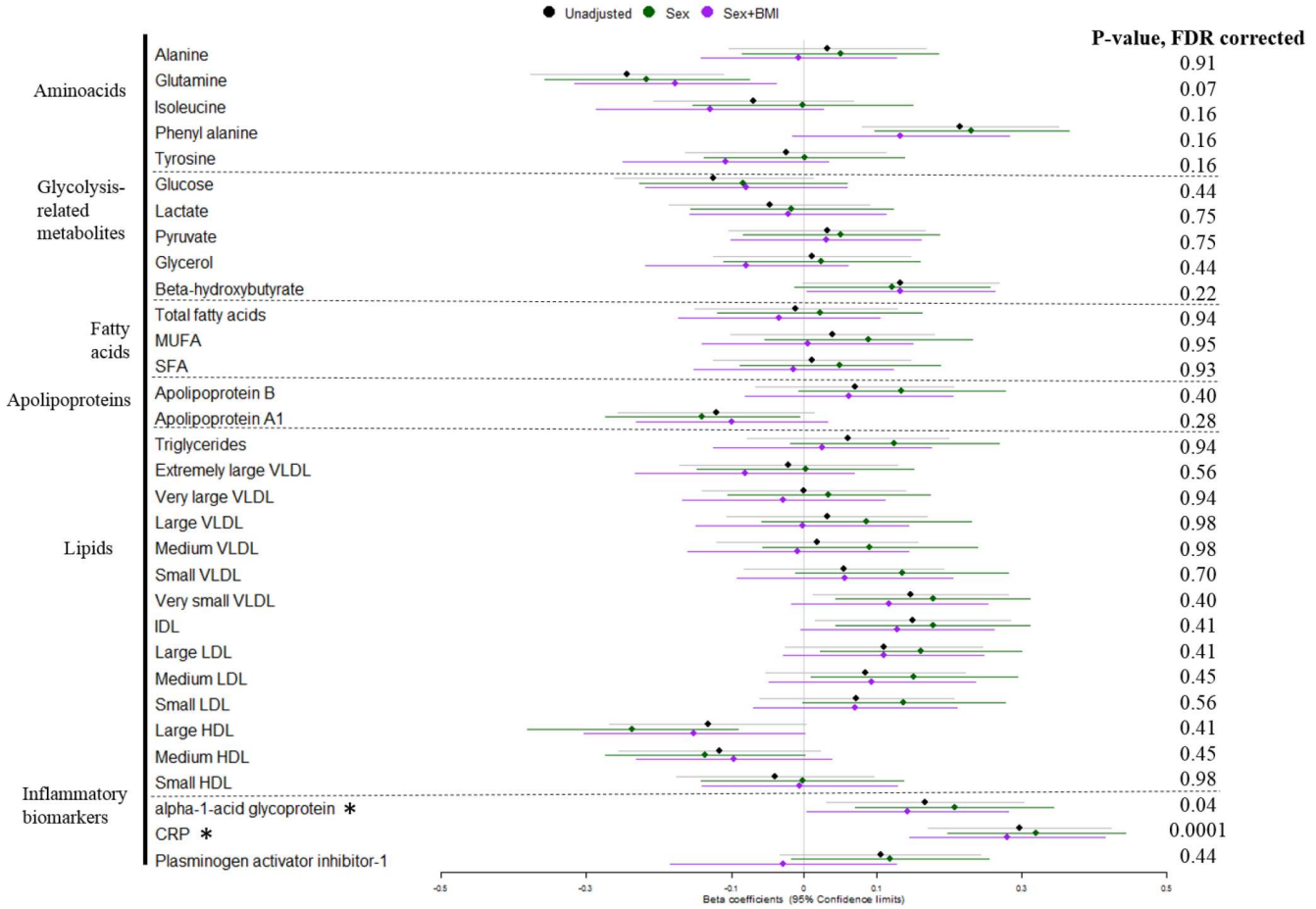
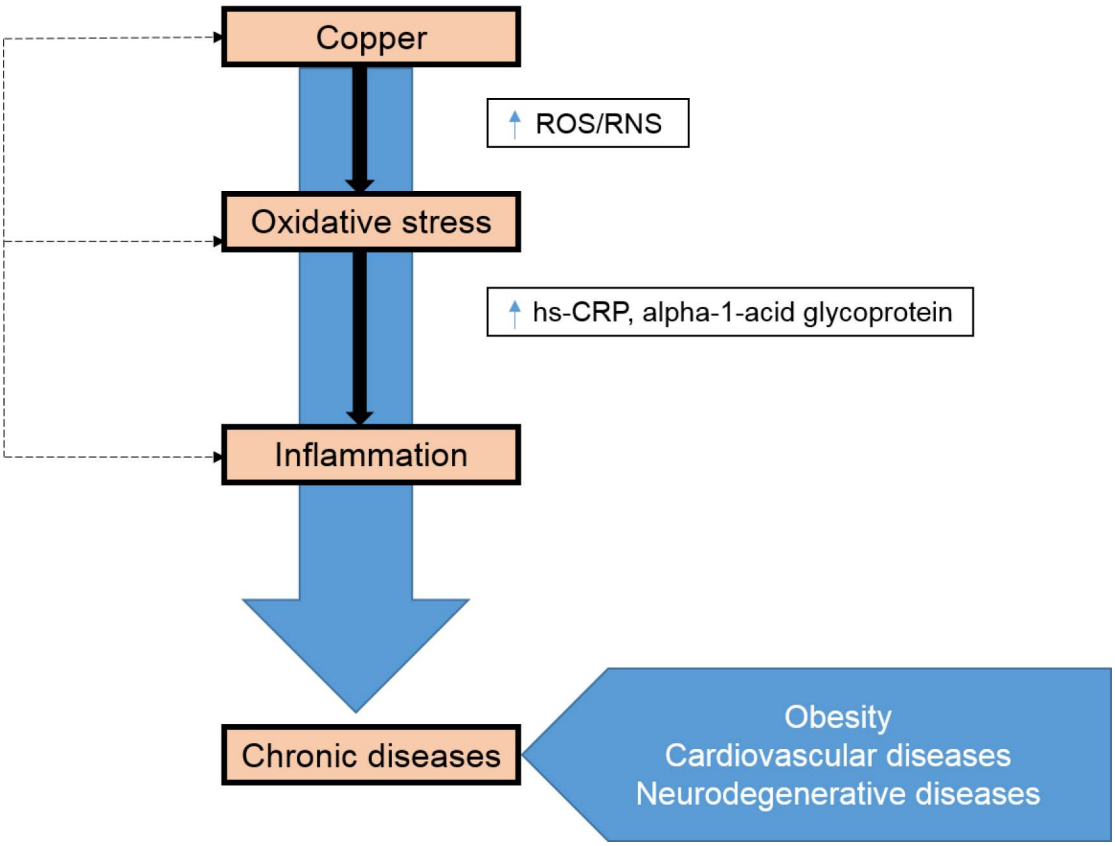


Figure 2 represents the relationship between copper and inflammation.



Legend for Figure 2: Copper and inflammation. Copper induces oxidative stress through imbalance in antioxidant system that increase reactive oxygen and nitrogen species (ROS, RNS), which might induce the activation of signaling pathways that upregulate proinflammatory cytokines leading to inflammation. However, inflammation is a hallmark of several chronic diseases including obesity, cardiovascular and neurodegenerative diseases. There are still controversies on causal and consequential interconnections between copper, oxidative stress and inflammation: those are mentioned throughout the text and represented by dotted lines. hs-CRP - high sensitivity C-reactive protein.