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Metal mixtures with longitudinal changes in lipid profiles: findings from the Manganese-exposed Workers Healthy Cohort

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Abstract

The majority of epidemiological investigations on metal exposures and lipid metabolism employed cross-sectional designs and focused on individual metal. We explored the associations between metal mixture exposures and longitudinal changes in lipid profiles and potential sexual heterogeneity. We recruited 250 men and 73 women, aged 40 years at baseline (2012), and followed them up in 2020, from the manganese-exposed workers healthy cohort. We detected metal concentrations of blood cells at baseline with inductively coupled plasma mass spectrometry. Lipid profiles were repeatedly measured over 8 years of follow-up. We performed sparse partial least squares (sPLS) model to evaluate multi-pollutant associations. Bayesian kernel machine regression was utilized for metal mixtures as well as evaluating their joint impacts on lipid changes. In sPLS models, positive association was found between manganese and change in total cholesterol (TC) (beta = 0.169), while negative association was observed between cobalt (beta = -0.134) and change in low density lipoprotein cholesterol (LDL-C) (beta = -0.178) among overall participants, which were consistent in men. Furthermore, manganese was associated with increased risks of incident hyper-cholesterolemia or hyper-LDL cholesterolemia (odds ratio: 1.849 or 2.354). Interestingly, rubidium was positively associated with change in LDL-C (beta = 0.273) in women, while copper was negatively associated with change in TC (beta = -0.359) and LDL-C (beta = -0.267). Magnesium was negatively associated with change in TC (beta = -0.327). We did not observe the significantly cumulative effect of metal mixtures with lipid changes. In comparison to other metals, manganese had a more significant influence in lipid changes (posterior inclusion probability = 0.559 for TC in men). Furthermore, male rats exposed to manganese (20mg/kg) had higher level of LDL-C in plasma and more apparent inflammatory infiltration, vacuolation of liver cells, nuclear pyknosis, fatty change than the controls. These findings highlight the potential role of metal mixtures in lipid metabolism with sex-dependent heterogeneity. More researches are needed to explore the underlying mechanisms.

1. Introduction

Dyslipidemia has experienced a remarkable increase in the last two decades, particularly in developing countries. Over the last decade, the prevalence of dyslipidemia of Chinese adults has risen from 18.6–40.4% (Huang et al. 2019). Based on the data from Global Burden of Disease Study in 1990–2019, elevation in low density lipoprotein cholesterol has risen from the 14th to the 8th leading risk factor of attributable disability-adjusted life-years worldwide (Collaborators 2020). New evidence has emerged and suggested the link between elevated triglyceride-rich lipoprotein and low-grade inflammation, atherosclerotic cardiovascular disease as well as all-cause mortality (Nordestgaard 2016). Unfavorable lipid trajectories throughout adulthood (35 years) were supposed to be related to a higher cardiovascular and mortality risk in later life (Duncan et al. 2019). There is an urgent need for exploring the pathogenesis for dyslipidemia, particularly the early and minor alteration in lipid profiles.

The roles of related factors have been well studied, such as diet and lifestyles, however, the impacts of exposures from environment remain understudied. Growing evidences emerged and suggested links between environmental factors, including metals, and alteration in lipid levels or dyslipidemia (Chen et al.

2020, Karim et al. 2013, Ledda et al. 2018, Yang et al. 2017, Yue et al. 2022, Zang et al. 2018, Zhou et al. 2016). The majority of previous studies were restricted by exposure to single metal and few cross-sectional researches have investigated relationships of multiple metals with dyslipidemia risk or metabolic syndrome components (Bulka et al. 2019, Moon 2014, Park &Kim 2019, Xu et al. 2020, Zhu et al. 2021). However, no conclusive results were drawn from the previous researches owing to potentially reverse causation by cross-sectional design or bias. Though several researches were available on the longitudinal associations of metal exposures with incident of dyslipidemia (Bai et al. 2015, Jiang et al. 2021, Kuo et al. 2018, Stranges et al. 2011, Xiao et al. 2019), scarce evidence was available on alteration in lipid profile.

It is known that people are faced with metal mixture exposures in reality. More evidences emerged suggesting the toxic effects of metal mixture exposures were not the same as that of a single metal (Silva et al. 2002, Wu et al. 2016). Therefore, we should pay more attention to the potential health effects related to metal mixture exposures in the development of dyslipidemia or alteration in lipid profile. Given sparse partial least squares (sPLS) regression can be used in dimension reduction and variable selection (Chun &Keles 2010), while Bayesian kernel machine regression (BKMR) can flexibly assess combined effects of mixture components (Bobb et al. 2015), we utilized sPLS and BKMR models to reduce dimension of metal mixtures and identify effect of metal mixtures on lipid change. Here we aimed to assess the associations of multiple metal exposures with changes in lipid profiles in the subjects from the manganese-exposed workers healthy cohort (MEWHC).

2. Materials And Methods

2.1 Study participants

The participants were gathered from the MEWHC. More detail on the cohort was provided in previous studies (Lv et al. 2014, Zhou et al. 2018). Participants (n = 573) were selected both in baseline (2012) and follow-up (2020) visits, whose information on demographics, lifestyles and physical examination were available. We excluded people who changed occupation type during the time interval (n = 164), lacked data on lipid profile (n = 30) or metal concentrations in blood cells (n = 43), and outliers with abnormal metal level [higher than three times percent 99 of metals (n = 13)]. Finally, 323 participants were remained in final analysis.

Written informed consent was signed by each participant, and this study was approved by the Ethics and Human Subject Committees of Guangxi Medical University.

2.2 Blood collection

After overnight fast, 5mL blood sample from peripheral venous was collected. Then the blood sample centrifugation was performed for separating plasma, serum and blood cells. The samples were refrigerated at − 80℃ prior to analysis.

2.3 Determinations of metal concentrations in blood cells

We measured 22 metal concentrations of blood cells following protocols (Xiao et al. 2021). The value, equaling to detection limit (LOD) divided by $\sqrt{2}$, was imputed for the sample whose concentration was below the corresponding LOD. The percentages of participants whose concentrations of tin, antimony and aluminum lower than LOD were higher than 20%. Therefore, we did not include these metals for further analysis.

2.4 Metal concentration variability assessments

We evaluated the metal concentration variability by comparing level in 2012, 2017 and 2020, respectively. More details were available elsewhere (He et al. 2022). We calculated correlation coefficients from Spearman's rank-order analysis and intraclass correlation coefficient (ICC) to assess variation across the visits. As a result, 10 metals with ICCs higher than 0.4 were remained in final analysis.

2.5 Determination of lipid parameters

Levels of lipid profiles, including triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were determined with an automatic biochemical analyzer (Hitachi 7600-020, Kyoto, Japan). The longitudinal change in lipid was defined as lipid level at follow-up minus lipid level at baseline. According to the diagnostic criteria in Chinese adults, categories of dyslipidemia were defined as follows: (1) hypertriglyceridemia (TG $\geq$ 1.7mmol/L); (2) hypercholesterolemia (TC $\geq$ 5.2 mmol/L); (3) hyper-LDL cholesterolemia (LDL-C $\geq$ 3.4 mmol/L); (4) hypo-HDL cholesterolemia (HDL-C < 1.0 mmol/L) (Hu 2017).

2.6 Definition of covariates

Socio-demographic characteristics and lifestyle habits were collected by face-to-face questionnaire. Seniority was defined as the length (year) of subjects worked for the ferro-manganese refinery. Current drinker (yes) drank at least once in a week for more than six months; Current drinker (no) were those who drank less than 5 ml at a time or never. Current smoker (yes) smoked at least one cigarette in a day for more than six months; current smoker (no) was the rest.

2.7 Animal experiments

Eighty Sprague-Dawley rats (3-week old, male and female in half, 125-150g, purchased from the Experimental Animal Center of Guangxi Medical University) were used, and procedures were as described in the previous study (Cheng et al. 2018). Male and female rats were randomly subdivided into 4 groups of 10 animals each, which were treated with 2 ml/kg of sterile saline, 5.0 mg/kg, 10.0 mg/kg, and 20.0 mg/kg of $MnSO_4 \cdot H_2O$ (St. Louis, MO, USA), via intraperitoneal injection for 24 weeks. On the next day after 24 weeks of $MnSO_4 \cdot H_2O$ treatment, the rats were sacrificed by cervical dislocation, plasma and liver were immediately excised and stored at -80°C.

Inductively coupled plasma mass spectrometry (ICP-MS) was employed to detect concentrations of hepatic manganese in rats. Briefly, approximately 50mg of a hepatic tissue from rat was transferred to a microwave cuvette and the exact mass was measured. Digestion was carried out by a microwave

accelerated reaction system (MARS6 Classic, GEM, USA) and samples were decomposed in 5 mL of nitric acid (65%, Merck, Germany) at 180°C, by applying the following temperature program: step1: 10 min warm-up to 120°C and heating for additional 5 min; step2: 15 min warm-up to 180°C and heating for additional 20 min. After cooling, decom-posed samples were transferred into the centrifuge tubes (Eppendorf, Germany) and diluted to 25 mL with ultrapure water (resistance of 18.2 MΩ, obtained from Cascada □.□ 10 system, PALL, USA). The method of quality control refers to the previous study (Stojsavljevic et al. 2020). We used the certified reference materials (Seronorm™ Trace Elements Whole Blood RUO no.210105, 210205, and 210305, SERO, Norway) and Standard Reference Material (SRM1640a, Trace Elements in Natural Water, National Institute of Standards and Technology, USA) as standard reagent for quality assurance in tissue measurement.

Lipid profiles in plasma of rats were measured with an automatic biochemical analyzer (Hitachi 7600-020, Kyoto, Japan). After fixing the tissue, peripheral organs from 3 animals of each group were transversally sectioned, dehydrated in ethyl alcohol (75%) and cleared by toluene, then embedded in paraffin wax and sectioned in 5μm slices. The slices were stained with hematoxylin and eosin for histological analysis. The samples were examined under a light microscope (ZEISS IMAGER A2-M2, Germany) and images were taken at 40X magnification. 2.8 Statistical analysis

2.8 Statistical analysis

We used mean (standard deviation) or frequency (percent) to present demographic information. In order to reduce skewed distributions of metal concentrations in blood cells, the metals were log10-transformed and represented as median [25th (*P*25), 75th percentiles (*P*75)]. Spearman's rank-order correlation analysis was performed for correlation assessment across the metals.

For single-metal model, linear regression model (ordinary least-squares, OLS) was performed to assess the relationships of 10 metals with lipid changes with the metal entering the model as a separated predictor. Gender and baseline age, seniority, BMI, smoking habits, drinking habits and lipid levels were adjusted for covariates. For multiple-metal model, the relationships of 10 metals with lipid changes adjusted covariates as in single-metal model and metals were simultaneously entered into the model.

In multi-pollutant model, sPLS regression was employed for assessing the relationships of metal mixtures with lipid changes. The number of latent components in the model (K) and thresholding parameter (eta) were determined by a 10-fold cross validation in the optimal model.

In BKMR model, we assessed the nonlinear and interactive effects as well as cumulative effects of 10 metals on lipid changes. First, we used posterior inclusion probabilities (PIP) analyses to assess the importance of metals on lipid changes. The univariate dose-response curve of specific metal with lipid change was showed when the remaining was fixed at *P*50. The difference (95% CI) in lipid change related to increase in metal concentration from its *P*25 to *P*75 was determined with the remaining metals fixed at *P*25, *P*50 or *P*75, respectively. The overall effects of metal mixtures at particular quantiles were estimated

in comparison to their *P*50s. We evaluated the interactions between metals on lipid changes. To investigate potential sexual heterogeneity, gender-stratified analysis was performed in all models.

We further applied the unconditional logistic regression analysis to calculate odds ratio (95%CI) for incident dyslipidemia in relation to metals in longitudinal analysis.

For animal experiments, tow-way ANOVA and LSD post-hoc tests were used to test the difference between manganese-exposed groups and the controls. All graphs were plotted using GraphPad Prism 6.

All statistical analyses were performed with R (Version 4.0.3), SPSS (version 26.0, IBM) and two-sides *P* value < 0.05 was considered as statistically significant.

3. Results

3.1 Characteristics of the participants

Table 1 summarizes the demographic characteristics and metal concentrations of 323 participants (men = 250, women = 73). The majority of participants were likely to be married or in cohabitation, with a normal BMI and high school education level or above. Current smoker and current drinkers were almost found in men. None of the women were current smokers and less was current drinkers. The lipid at followed-up were significantly higher than that at baseline except HDL-C (all $P < 0.001$) (Fig.S1).

Table 1
The baseline characteristics and levels of metals in blood cells among the 323 participants from the MEWHC.

Variables[a]	Overall	Men	Women	*P*
	(n = 323)	(n = 250)	(n = 73)	
Age, years	40 (6.1)	40 (6.5)	38 (4.5)	0.025[b]
Seniority, years	16 (7.9)	17 (8.2)	12 (5.7)	**< 0.001[b]**
BMI, kg/m^2	23 (3.0)	23 (3.0)	22 (2.9)	**0.044[b]**
Ethnicity				0.120[c]
Han	143 (44.0)	103 (41.0)	40 (55.0)	
Zhuang	166 (51.0)	135 (54.0)	31 (42.0)	
Other	14 (4.0)	12 (5.0)	2 (3.0)	
Education				**0.028[c]**
Middle school or lower	143 (44.0)	102 (41.0)	41 (56.0)	
High school or higher	180 (56.0)	148 (59.0)	32 (44.0)	
Marital status				**0.012[c]**
Single/widow	24 (7.0)	24 (10.0)	0 (0.0)	
Married/cohabited	299 (93.0)	226 (90.0)	73 (100.0)	
Current smoker				**< 0.001[c]**
Yes	194 (60.0)	194 (78.0)	0 (0.0)	
No	129 (40.0)	56 (22.0)	73 (100.0)	
Current drinker				**< 0.001[c]**
Yes	234 (72.0)	216 (86.0)	18 (25.0)	
No	89 (28.0)	34 (14.0)	55 (75.0)	
Mg, mg/L	59.38 (54.36, 64.08)	60.23 (55.29, 64.30)	55.3 (49.44, 60.68)	**< 0.001[b]**

Variables[a]	Overall	Men	Women	*P*
	(n = 323)	(n = 250)	(n = 73)	
Mn, μg/L	29.30 (24.13, 36.26)	28.02 (22.67, 34.92)	33.80 (28.47, 43.54)	**< 0.001[b]**
Fe, mg/L	1058.52 (996.08, 1111.10)	1065.23 (1001.07, 1116.96)	1039.33 (956.29, 1089.14)	**0.018[b]**
Co, μg/L	0.09 (0.07, 0.12)	0.08 (0.07, 0.10)	0.13 (0.10, 0.24)	**< 0.001[b]**
Cu, μg/L	780.68 (731.39, 861.34)	772.00 (730.98, 841.20)	837.54 (731.60, 919.95)	**0.007[b]**
Zn, mg/L	10.02 (9.08, 10.98)	10.01 (9.18, 11.00)	10.05 (8.96, 10.91)	0.691[b]
Se, μg/L	211.39 (189.54, 239.50)	210.23 (188.74, 237.93)	224.76 (191.81, 246.71)	0.144[b]
Rb, mg/L	5.79 (5.28, 6.32)	5.82 (5.30, 6.41)	5.63 (5.09, 6.02)	**0.006[b]**
Cd, μg/L	5.34 (2.51, 11.23)	7.67 (2.53, 12.39)	3.76 (2.48, 4.98)	**< 0.001[b]**
Pb, μg/L	142.28 (92.65, 209.54)	161.81 (115.59, 228.08)	85.44 (64.17, 134.69)	**< 0.001[b]**

Note: [a] Continuous variables were presented as mean (SD) or median (25th, 75th percentiles). Categorical variables were

presented as n (%).

[b]Mann-Whitney U test was used for continuous covariates across the different groups.

[c]Chi-square test for categorical covariates across the different groups.

Abbreviations: MEWHC, manganese-exposed workers healthy cohort; BMI, body mass index. Mg, magnesium; Mn, manganese; Fe, iron; Co, cobalt; Cu, copper; Zn, zinc; Se, selenium; Rb, rubidium; Cd, cadmium; Pb, lead.

3.2 Metal concentrations and corresponding correlations

The metal concentrations in blood cells are showed in Table 1. Highest of median concentration was observed for iron (1058.52 mg/L), and lowest was observed for cobalt (0.09 μg/L). The concentrations of rubidium, cadmium and lead in men were higher as compared to women, while manganese, cobalt, copper were opposite (all $P < 0.05$).

We also performed spearman's rank-order correlations analysis to reveal the correlations between the metals in blood cells (Fig. 1).

3.3 Associations of metals in blood cells with lipid changes in single-metal model

In overall, manganese showed a positive association with TC changes (beta = 0.808); Cobalt was negatively associated with LDL-C changes (beta = -0.518). For men, manganese showed a positive association with TC changes (beta = 0.899); Cobalt was negatively associated with LDL-C changes (beta = -0.725). However, we found no significant relationship between metal and lipid changes for women (Table S1);

3.4 Associations of metals in blood cells with lipids changes in multi-metal model

In overall, manganese showed a positive association with TC changes (beta = 0.909) while cobalt showed a negative association with TC changes (beta = -0.636). A negative association was found for cobalt and LDL-C changes (beta = -0.716). In gender-stratified analysis, a negative association was found for zinc and TC changes only in men (beta = -1.947). Cobalt showed a negative association with LDL-C changes in men (beta = -0.813). Copper was negatively associated with TC changes only in women (beta = -7.304) (Table S2);

3.5 Associations of metals in blood cells with lipid changes in sPLS model

In sPLS regression model, we selected predictive metals with lipid changes with adjustment for covariates. The optimal model was selected for the minimum mean squared prediction error according to the 10-fold cross-validation. The error path and the selected metals were available in Fig.S2 and Table 2, respectively. In overall participants, we observed positive association between manganese (beta = 0.169) and TC change, whereas a negative association were established between cobalt (beta = -0.178) and LDL-C change (both $P < 0.05$). In men, the association of manganese (beta = 0.156) with TC and cobalt with LDL-C change (beta = -0,133) were in line with that in overall (both $P < 0.05$). In women, magnesium (beta = -0.327) and copper (beta = -0.359) showed negative associations with TC change and rubidium (beta = 0.273) showed a positive association with LDL-C change.

Table 2
Associations between metals in blood cells and changes in lipid profiles: estimated change from multi-pollutant models using sPLS.

		beta(95%CI)			beta(95%CI)
Overall			Women		
TC			TC		
	Co	**-0.134 (-0.267, -0.005)**		Mg	**-0.327 (-0.590, -0.066)**
	Mn	**0.169 (0.068, 0.279)**		Co	-0.291 (-0.678, 0.053)
TG				Cu	**-0.359 (-0.660, -0.039)**
	Rb	0.084 (-0.028, 0.189)		Mn	0.302 (-0.038, 0.654)
LDL				Rb	0.142 (-0.147, 0.371)
	Co	**-0.178 (-0.294, -0.071)**		Se	0.151 (-0.150, 0.395)
	Mn	0.086 (-0.027, 0.200)	TG		
				Rb	0.111 (-0.050, 0.273)
Men				Zn	0.136 (-0.023, 0.283)
TC			LDL		
	Co	-0.073 (-0.207, 0.062)		Mg	-0.196 (-0.467, 0.047)
	Mn	**0.156 (0.034, 0.274)**		Co	-0.220 (-0.614, 0.057)
	Se	0.11 (-0.024, 0.246)		Cu	**-0.267 (-0.506, -0.027)**
	Zn	-0.132 (-0.247, 0.005)		Mn	0.227 (-0.067, 0.563)
TG				Rb	**0.273 (0.011, 0.512)**
	Mg	0.082 (-0.053, 0.198)			
	Mn	-0.066 (-0.190, 0.052)			
	Rb	0.065 (-0.065, 0.186)			
LDL					
	Co	**-0.133 (-0.245, -0.033)**			

beta(95%CI)	beta(95%CI)

Estimates are presented as the raw coefficients obtained from sPLS regression analyses.

Models are adjusted for age, and/or gender, work duration, BMI, education, ethnicity, marital status, smoking status, drinking status, TG, TC, HDL-C, LDL-C;

Abbreviations: sPLS, sparse partial least-squares; BMI, body mass index; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Mg, magnesium; Mn, manganese; Fe, iron; Co, cobalt; Cu, copper; Zn, zinc; Se, selenium; Rb, rubidium; Cd, cadmium; Pb, lead.

Table 3 Adjusted odds ratios (95% CI) of incident dyslipidemia associated with metal levels in overall.

Dyslipidemia	N (case/control); %	Metals	OR (95% CI)	*P* value
Hypercholesterolemia	84/157; 34.9%			
		Mn	1.849 (0.101, 3.597)	**0.038**
		Co	-0.744 (-2.146, 0.658)	0.298
Hyer-LDL cholesterolemia	36/248; 12.7%	Mn	2.354 (-0.025, 4.733)	0.052
		Co	-2.508 (-5.081, 0.065)	0.056

Model was adjusted for age, gender, BMI, seniority, education, smoking status, drinking status and baseline lipid levels.

Abbreviations: Mn, manganese; Co, cobalt; hyper-LDL cholesterolemia, hyper-low-density lipoprotein cholesterolemia;

3.6 Bayesian kernel machine regression analysis

No significant cumulative effect of the metal mixture was observed on changes for TC, TG or LDL-C (Fig. 2). To investigate the dose-response relationship, we estimated the univariate relationship of specific metal with changes in lipid markers with other metals fixed at *P*50 (Fig.S3). The results indicated dose-response relationships between specific metal and changes in lipid markers appeared to be approximately linear. We then sought to learn the difference (95%CI) of lipid changes caused by specific metal increasing from its *P*25 to *P*75 with the remaining fixed at *P*25, *P*50 or *P*75, respectively. We observed manganese displayed a significantly positive association with TC in overall participants (Fig.S4A). We also detected potential interaction effects but there was little evidence for interactions between the metals on changes in lipid profile (Fig.S5).

3.7 Associations of metals with dyslipidemia

Further, we performed the unconditional logistic regression analyses among all participants to assess the impacts of metals selected in the sPLS models on the risk of incident dyslipidemia. Adjusted odds ratios (95%CI) of manganese with hypercholesterolemia was 1.849 (0.101, 3.597); Manganese was positively while cobalt was negatively associated with risk of hyper-LDL cholesterolemia (odds ratios, 2.354 *vs.* -2.508), respectively, though the significance were at borderline. No statistically significant association was detected between cobalt and hypercholesterolemia or hyper-LDL cholesterolemia (Table 3).

3.8 Associations of chronic manganese exposure and lipid profile in rats

To further verify the association between manganese exposure and lipid profile, we firstly detected the concentration of hepatic manganese in rats (Fig.S6). When compared with the control group, only males that treated with 20 mg/kg MnSO4 were observed a significantly higher level of manganese ($P = 0.002$), and there was a significant difference in manganese deposition between sexes at this dose. In addition, only the plasma LDL-C levels significantly increased in male rats that received the highest dose ($P = 0.026$). None significant difference was found in lipid levels between the groups of females (Fig.S7).

Finally, the liver histopathological analysis was pretended in Fig.S8. No major abnormalities were observed in the control group. Rats that received 5 mg/kg $MnSO_4$ showed minor sinusoidal dilatation with slightly inflammatory infiltrates in both sexes. The medium and high-dose group rats presented apparent inflammatory infiltration, vacuolation of liver cells, nuclear pyknosis, fatty changes, and fibrosis (Fig.S8).

4. Discussion

The strongest finding in the present study is to identify of sex-specific metals related to lipid changes among the participants from MEHWC. The consistent findings from sPLS and BKMR analysis indicated a significantly positive association of manganese with elevated TC and LDL-C among overall participants and men. Furthermore, manganese was associated with increased risk of incident hyper-cholesterolemia and hyper-LDL cholesterolemia (odds ratio: 1.849 *vs.* 2.354). The BKMR model showed manganese dominated the positive cumulative effect of ten metal mixtures on elevated TC and LDL-C, though the effects including the null. Interestingly, we found a significantly negative association of copper with elevated TC and LDL-C among women.

In addition to sex-difference in metal level, previous researches have also revealed sex differences in lipid levels or lipid patterns. Higher risk of cardiovascular disease was observed in men compared with women, even if with comparable serum lipid concentrations (Johnson et al. 2004). It was reported higher TC and HDL-C were observed in women from the Multi-Ethnic Study of Atherosclerosis study (Goff et al. 2006). Moreover, higher large HDL-C, large HDL-C to total HDL-C ratio and less small HDL-C were found in women in comparison to men (Johnson et al. 2004). Whilst men have a higher fraction of small dense LDL-C and larger VLDL particles, and small dense LDL-C has been implicated as a major cardiovascular disease risk factor. Based on the sex-difference in the metals and lipids, we performed the stratified

analysis by sex. Interestingly, we found some associations between metal exposure and change in lipid profile with sex-specific heterogeneity.

By selecting the predictive metals associated with lipid changes, our study may provide additional clinical value to identify individuals with elevated lipid profile. The use of sPLS model helped to identify the associations between metals and lipid changes, which might be covered up by high correlation or data dimension in traditional statistical models, such as copper and rubidium for LDL-C in women and cobalt for TC in overall participants. Furthermore, the importance of each metal on the lipid change was quantified accounting for inter-metal interactions. As showed in PIP analysis, manganese might play the most important role in association of metal mixture exposures with change in TC in men, given the highest PIP (0.559) among metals. It is interesting given that the association of manganese with lipid change was independent, suggesting the complexity in relationship may not be noticed in conventional analysis strategy. However, manganese serves as both essential metals and neurotoxins depending on doses (Li &Yang 2018). The variation in the shape of relationship may weaken the overall association with change of lipid profile. Moreover, MEWHC was performed among occupational workers in China, the harmful effects of heavy metals might be less profound among general population.

Moreover, our study assessed the potential sexual heterogeneity in the association of manganese with lipid changes. For instance, positive association was found between manganese and change in lipid in men but not women. Oxidative stress leading to lipid peroxidation is a well-known mechanism for manganese toxicity, and manganese imbalance may promote more reactive oxygen species (ROS) producing, leading to oxidative stress, inflammation and endothelial dysfunction (Bornhorst et al. 2013). An animal experiment found that manganese enhanced cholesterol biosynthesis in the rats' liver microsome, and stimulated farnesyl pyrophosphate synthase activity, which was an important synthesis pathway for regulating cholesterol biosynthesis and metabolism (Bell &Hurley 1973). Differences in response to hormones between men and women might account for the association of manganese with lipids changes. Manganese was positively associated with sex hormone binding globulin (SHBG) (Rotter et al. 2016). Previous researchers indicated higher SHBG level showed significant relation to an elevated risk of metabolism syndrome among men (Bhasin et al. 2011, Haring et al. 2013). The interactions in manganese and SOD, SHBG and other sex hormones may involve in this. Sex difference in smoking habit may be a potential explanation. Higher serum manganese levels were observed in smokers in comparison to non-smokers (Ates Alkan et al. 2019). In this study, current smokers accounted for a higher proportion in men, which may provide an interpretation for association with sex specific. The presence of sex-specific associations and the physiological mechanisms behind warrants further investigation.

Interestingly, a negative association between copper and change in TC (beta = -0.359) and LDL-C (beta = -0.267) was observed only in women. In line with the previous research, higher copper concentration were observed in women in comparison to men (Helgeland et al. 1982). Differences in diet and copper absorption between men and women as well as the effects of estrogen on copper metabolism (Songchitsomboon &Komindr 1996) might provide an explanation. Furthermore, evidence emerged from cross-sectional epidemiological study and showed serum copper of subjects with normal levels of low-

density lipoprotein cholesterol was significantly lower in comparison to those who having high levels of low-density lipoprotein cholesterol in women (Ghayour-Mobarhan et al. 2009). In addition, copper ions showed activity to activate cholesterogenic genes in macrophages, underlying the potential mechanism of atherosclerosis related with copper (Svensson et al. 2003). Furthermore, a recent cross-sectional study of our team observed copper in serum was inversely associated with estradiol (Zan et al. 2021). Estrogen-related receptor alpha (ERRα) acts downstream of substantial sex differences in lipid metabolism, and endogenous estrogen plays a estrogen/ERα signaling in contributing to the sex-difference in hepatic VLDL secretion affecting hepatic lipid homeostasis. Moreover, hepatocyte-specific ERα-knock-out mice has lost the ability of estrogen to diminish liver fatty degeneration, which indicated hepatic estrogen directly decrease lipid accumulation via ERα. Lack of hepatocyte ERα leads to lack of estrogen regulation of target genes, increased expression of lipid synthesis genes, and impaired estrogen-regulation of other lipid metabolic target genes.

The study is the first to evaluate the associations of metal mixture exposures with lipid change by a prospective longitudinal study. In addition, we used different methods (sPLS and BKMR models) to remedy the gaps in traditional approaches, and we observed consistent findings. However, there were several limitations in our study. First, the sample size was relatively small, limiting the power for interpretation of results, particularly in women. Second, baseline levels of metals might not completely represent the exposure level in a long term. However, metals levels could be considered to be relatively stable because the occupation type of the participants were fixed; Moreover, metals in blood cells showed fair reproducibility (ICC > 0.4). Last but not the least, this study was based on an occupational population who were exposed to relatively high level of metals, and the findings need to be further confirmed in the general population.

5. Conclusions

To conclude, these findings highlight the potential role of metal mixtures in lipid metabolism with sex-dependent heterogeneity. More researches are needed to explore the underlying mechanisms.

Declarations

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Authors' contributions

Xiaoting Ge: Writing - original draft, Writing - review & editing. **Guohong Ye:** Writing - original draft, Writing - review & editing. **Junxiu He:** Conceptualization, Writing - review & editing. **Yu Bao:** Investigation, Data curation. **Yuan Zheng:** Investigation, Data curation. **Hong Cheng:** Methodology. **Xiuming Feng:**

Investigation, Data curation. **Wenjun Yang:** Investigation, Data curation. **Fei Wang:** Writing - review & editing. **Yunfeng Zou:** Conceptualization. **Xiaobo Yang:** Supervision, Project administration.

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Availability of data and materials The datasets used or analyzed and materials during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Competing interest The authors declare that they have no competing interests.

Ethical approval The Medical Ethics Committee of Guangxi Medical University (ID: 20200021) have approved all study procedures.

Consent to participate All participants have signed the informed consents for this study.

Consent for publication The manuscript is approved by all the authors for publication.

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Figures

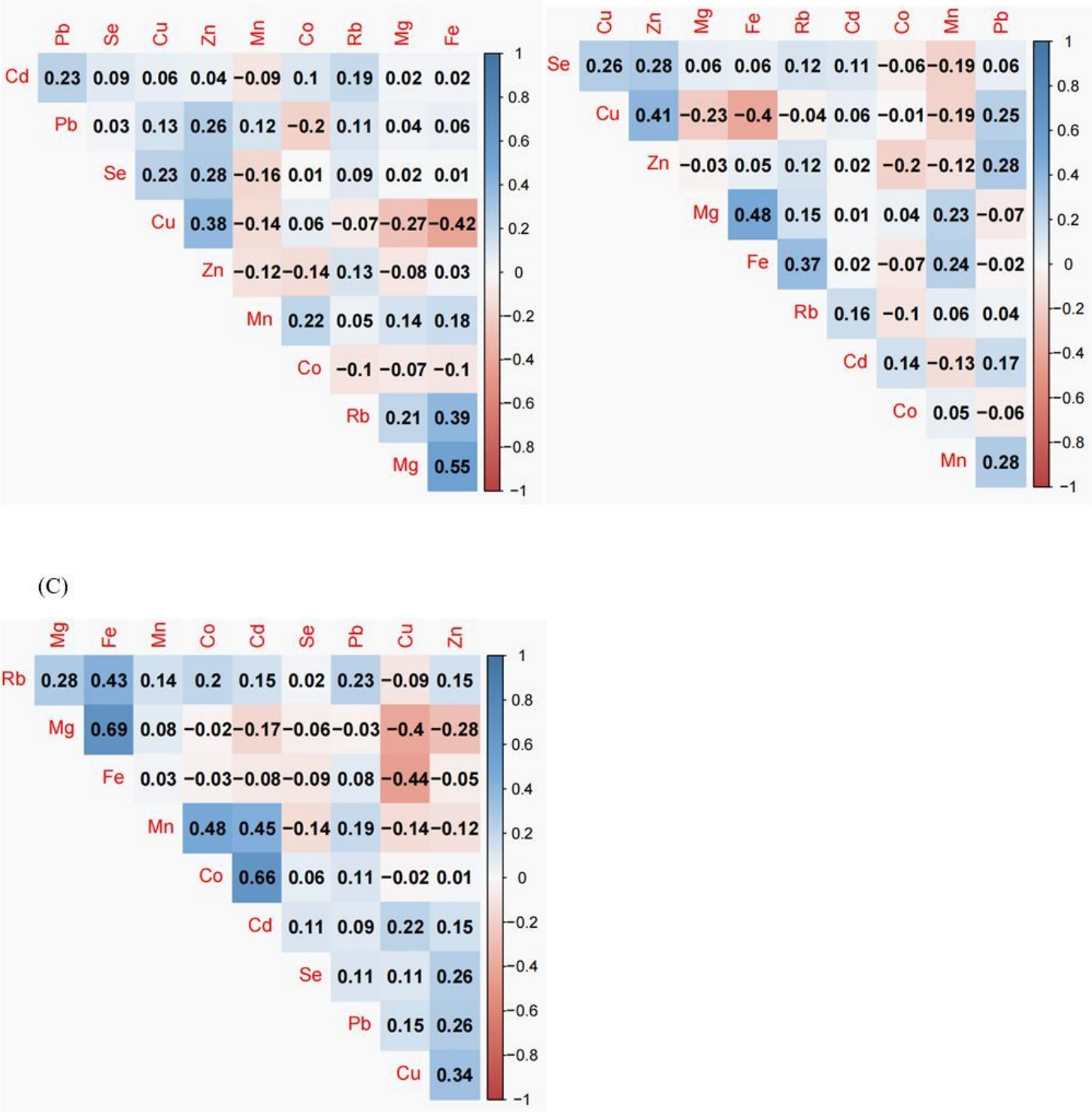

Figure 1

The correlations between the metals in overall (A), men (B) and women (C).

Abbreviations: Mg, magnesium; Mn, manganese; Fe, iron; Co, cobalt; Cu, copper; Zn, zinc; Se, selenium; Rb, rubidium; Cd, cadmium; Pb, lead.

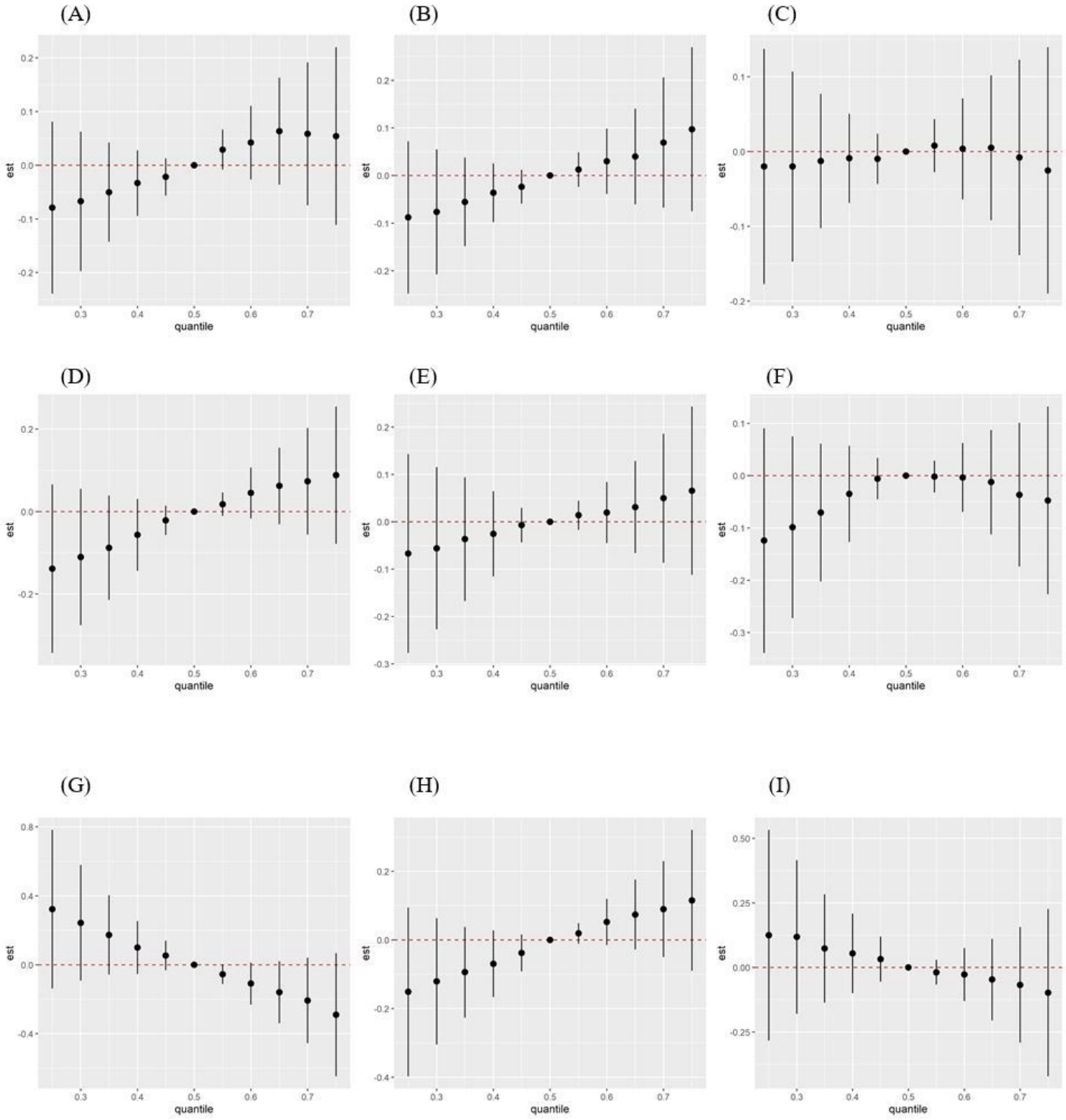

Figure 2

Cumulative effects of metal mixtures for changes in lipid profile in BKMR model. In overall participants, **(A)** TC**; (B)** TG**; (C)** LDL-C**;** In men, **(D)** TC**; (E)** TG**; (F)** LDL-C**;** In women, **(G)** TC**; (H)** TG**; (I)** LDL-C**;**

Abbreviations: TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol;

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