Associations between blood and urinary manganese with risk of metabolic syndrome and its components: cross-sectional analysis of National Health and Nutrition Examination Survey 2011-2016

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1   **Introduction**

2   Manganese (Mn) is an essential element in the human body that is mainly obtained

3   from water, nuts, grains, fruits, green vegetables, and caffeinated drinks (Gong et al.,

4   2020), which acts as a co-factor of superoxide dismutase (SOD), an enzyme responsible

5   for the degradation of superoxide radicals (Pfalzer and Bowman, 2017). Mn deficiency

6   may increase oxidative stress by producing more reactive oxygen species (ROS) (Li

7   and Yang, 2018), leading to inflammation and endothelial dysfunction (Liu et al., 2007;

8   Song et al., 2007), and accelerates the proliferation of vascular cells and increases

9   vasoconstriction (Tanito et al., 2004; Ward and Croft, 2006). Evidence from in vitro and

10   animal studies demonstrated that Mn supplementation could downregulate ROS

11   generation (Juttukonda et al., 2017), prevent endothelial dysfunction (Burlet and Jain,

12   2013) and reduce the levels of serum inflammatory biomarkers (Owumi and Dim, 2019).

13   However, excessive exposure of Mn from polluted air and water may lead to impaired

14   cognitive development and Parkinson's disease, especially among workers and general

15   populations residing near factories (Pfalzer and Bowman, 2017; Li and Yang, 2018).

16   Given the roles of Mn in alleviating oxidative stress, it may improve

17   cardiometabolic health with its anti-oxidative ability. Metabolic syndrome (MetS) is a

18   clustering of metabolic disorders, namely elevated waist circumference, impaired

19   glucose metabolism, elevated blood pressure and dyslipidemia (Expert Panel on

20   Detection, 2001), and is one of the precursors of various chronic diseases, including

21   diabetes, dementia, stroke and coronary heart disease (Pal et al., 2018; Saklayen, 2018).

22   When compared with other metal exposures, such as cadmium (Cd), lead (Pb), and

23   mercury (Hg) (Planchart et al., 2018; Ma et al., 2020), epidemiological evidence on the

24   relationship between Mn and MetS is limited and the results are inconsistent (Choi and

25   Bae, 2013; Li et al., 2013; Rhee et al., 2013; Rotter et al., 2015; Zhou et al., 2016; Bulka

26   et al., 2019). Most previous studies have used dietary methods to estimate Mn exposure.

27   In the analysis of Chinese National Nutrition and Health Survey 2010–2012 (Zhou et

28   al., 2016), dietary Mn was inversely associated with the odds of MetS, abdominal

29   obesity and elevated triglycerides among men, but positively associated with MetS and

30   low high-density-lipoprotein cholesterol (HDL-C) among women. In the analysis of the

31   Korea National Health and Nutrition Examination Survey (KNHANES) 2007–2008

32   (Choi and Bae, 2013), dietary Mn was lower among women with high blood pressure

33   but no significant difference among men. For another case-control study (Li et al., 2013),

34   dietary Mn was inversely associated with the odds of MetS and the number of MetS

35   components, but dietary assessment may subject to recall bias due to its self-reported

36   nature (Choi and Bae, 2013; Li et al., 2013; Zhou et al., 2016). Moreover, some studies

37   that have assessed Mn in urine or blood samples (Rhee et al., 2013; Rotter et al., 2015;

38   Bulka et al., 2019). A cross-sectional study in Poland found that serum Mn had

39   significant positive correlations with body mass index, waist circumference (WC),

40   waist-to-hip ratio, insulin, and insulin resistance, but no correlation with MetS (Rotter

41   et al., 2015). In analysis of KNHANES 2008 (Rhee et al., 2013), Mn from whole blood

42   or urine did not have significant relationship with MetS. A recent cross-sectional

43   analysis of the United States (U.S.) NHANES 2011–2014 did not find significant

44   association between Mn from whole blood with the prevalence of MetS, nor for the

45   methylmercury-manganese pattern using principal components analysis (Bulka et al.,

46   2019). Furthermore, our previous analysis among the U.S. NHANES participants has

47   found positive linear relationships between urinary Mn with fasting plasma glucose

48   among women, and J-shaped dose-response relationships of blood Mn with insulin

49   resistance among men (Yang et al., 2020). Although some analyses explored the

50   combined effects of metal including Mn on cardiometabolic health, there is a need to

51   investigate associations of Mn with MetS in a sex-specific manner.

52   To address the knowledge gap as mentioned above, we have analyzed the

53   NHANES data from a nationwide sample in the U.S. to investigate the overall and sex-

54   specific associations of urinary and blood Mn levels with the prevalence of MetS and

55   its components. We also explored the shape of dose-response relationships across the

56   doses of urinary and blood Mn levels and the prevalence of MetS.

57

58   **Materials and methods**

59   **Study population**

60   The U.S. NHANES program refers to multiple cross-sectional surveys conducted

61   among general population in the U.S. to collect data on diet, nutritional status, health

62   status and health behaviors of the participants. In the present study, we included data (n

63   =29,902) from surveys conducted in 2011-2012, 2013-2014, and 2015-2016 cycles. A

64   random subsample was performed for urinary and blood Mn, and other metal assays

65   during the three cycles (Centers for Disease Control and Prevention, 2011). We

66 included participants separately for analysis, the only difference in inclusion criteria

67 was the data availability of metals from urine or whole blood. For analyzing the

68 relationship between urinary Mn and MetS, we excluded participants who were missing

69 urinary metals data (n = 21,707), with missing value in biomarkers related to MetS (n

70 = 5,539), aged <18 years (n = 385), and with gestational diabetes mellitus (n = 1) or

71 gestational hypertension (n = 2). Since cardiovascular disease might influence the

72 association of urinary Mn levels with MetS, we further excluded participants with type

73 2 diabetes (T2D), coronary heart disease and stroke based on self-reported medical

74 history (n=556). Finally, 1,713 participants were included in urinary Mn analyses

75 (**Figure S1**). For analyzing the relationship between blood Mn and MetS, we applied

76 the same exclusion criteria and included 3,335 participants in the final analyses (**Figure**

77 **S1**).

78 **Measurements of Mn and other metals**

79   With the use of inductively coupled plasma mass spectrometers (ELAN® 6100

80 DRC^Plus or ELAN® DRC II, PerkinElmer Norwalk, Fairfield), 15 metal levels in urine,

81 namely Cobalt (Co), Mn, Cd, Hg, Arsenic (As), Pb, Molybdenum (Mo), Barium (Ba),

82 Cesium (Cs), Antimony (Sb), Tin (Sn), Strontium (Sr), Thallium (TL), Tungsten (W)

83 and Uranium (U), along with 5 metal levels in whole blood, namely Cd, Hg, Pb,

84 Selenium (Se) and Mn were measured. Spot urine and fasting blood sample were

85 collected at the time of the laboratory exam, shipped on dry ice to the National Center

86 for Environmental Health (NCEH) in Atlanta, GA, and stored frozen at −20 °C until

87 assayed (Centers for Disease Control and Prevention, 2011).

88   For urinary and blood metals that had levels below the detection limit, the values

89   were substituted with the detection limit divided by the square root of two. If the

90   detection limit differed between the three survey cycles, we selected the highest value

91   of the three cycles. The NHANES quality assurance and quality control (QA/QC)

92   protocols have met the 1988 Clinical Laboratory Improvement Act mandates (Bachner

93   and Hamlin, 1993a, b).

MetS Definition

95   MetS was defined according to the diagnostic criteria proposed by the Adult

96   Treatment Program III of the National Cholesterol Education Program (Expert Panel

97   on Detection, 2001). Participants with three or more of the following conditions were

98   classified as having MetS: 1) elevated triglyceride (TG) ≥ 1.69 mmol/L (150 mg/dL);

99   2) low HDL-C : men < 1.04 mmol/L (40 mg/dL), women < 1.29 mmol/L (50 mg/dL);

100   3) elevated fasting plasma glucose (FPG): ≥6.1 mmol/L (110 mg/dL); 4) elevated WC:

101   men ≥ 102 cm, women ≥ 88 cm; 5) elevated systolic blood pressure (SBP) ≥ 130 mmHg

102   and/or elevated diastolic blood pressure (DBP) ≥ 85 mmHg. Fasting blood samples

103   were examined in the morning session after a 9-hour fast, while blood pressure was

104   measured by physicians for three times to calculate the average value (Centers for

105   Disease Control and Prevention, 2011).

Covariates

107   Standardized questionnaires were used to obtain socio-demographics, lifestyle,

108   clinical, and nutritional factors from participants. We included covariates based on the

109   priori knowledge on the risk factors of adverse cardiometabolic health, including low

110 socioeconomic status (Ross et al., 2020), severity of depression (Godin et al., 2019),

111 smoking status (Adisen et al., 2018), excessive daily energy intake (Xu et al., 2018),

112 alcohol drinking status (Adisen et al., 2018), and the history of cancer (Bishehsari et al.,

113 2020). We included age (18-39, 40-59 or $\geq$60 years), sex (men or women),

114 race/ethnicity (Non-Hispanic White, Non-Hispanic Black, other Hispanic or other race),

115 educational attainment (less than high school, high school, at least some college),

116 poverty income ratio (PIR, dichotomized as <1 or $\geq$1), smoking status (categorized as

117 never, former or current smoker), dietary energy intake in quartiles, status of alcohol

118 drinking (dichotomized as yes or no), depression as classified by Patient Health

119 Questionnaire-9 (categorized as no depression, mild depression, moderate to severe

120 depression) (Patel et al., 2019), and self-reported history of cancer. Poverty-income

121 ratio (PIR) is the ratio of the family's self-reported income to the family's appropriate

122 poverty threshold according to the U.S. Census Bureau, and PIR values of 1.00 or

123 greater indicating people above the poverty threshold (Tyrrell et al., 2013). To calculate

124 daily energy intake, participants reported the food and beverage items reported

125 consumed between midnight and midnight 24 hours prior to the NHANES dietary

126 interview (Marriott et al., 2019).

127 **Statistical analysis**

128 Descriptive statistics were used to present the frequency and proportion of the

129 demographic. We compared the sex differences in participants' characteristics by chi-

130 square test. Urinary Mn was divided by urinary creatinine to control the concentration

131 dilution of urine. To account for the collinear nature of 15 urinary metals being

132   measured, we firstly conducted elastic net penalty regression to select the potential

133   metals that associated with MetS (Stafoggia et al., 2017; Hou et al., 2019). The elastic

134   net model can perform selection while enabling the inclusion of collinear predictors

135   through combining the least absolute shrinkage and selection operator and ridge (Zhao

136   et al., 2020). A set of elastic net coefficients were estimated and selected the metals that

137   their elastic net coefficients were not shrunken to zero. (Zhao et al., 2020)

138   Urinary and blood Mn levels was treated as categorical variable (classified as

139   quartiles, Q1 to Q4) or continuous variable (log10-transformed to reduce skewness).

140   Logistic regression models were used to examine the associations between urinary Mn

141   levels and the odds of MetS and each of its components (elevated TG, low HDL-C,

142   elevated FPG, elevated WC, elevated SBP and elevated DBP). The regression models

143   were adjusted for age, sex, race, education attainment, poverty income ratio, smoking

144   status, status of alcohol drinking, dietary energy, severity of depression, self-reported

145   history of cancer and urinary metals (all categorized in quartiles) as selected by elastic

146   net regression (multi-metal model). Tests of linear trend across increasing quartiles (Q)

147   of urinary Mn levels were performed by assigning the median levels in quartiles and

148   being treated as a continuous variable. Sensitivity analysis was performed to examine

149   the relationship between urinary Mn and MetS. We have added creatinine as a separate

150   covariate into regression models and used urinary Mn as exposure without dividing it

151   by creatinine (Barr et al., 2005).

152   The analysis plan when using blood Mn as exposure was the same as urinary Mn

153   except for not including urinary creatinine, with all blood metals, namely Cd, Hg, Pb,

154 Se (all categorized in quartiles) being included into the logistic regression model (multi-

155 metal model). Collinearity between Mn (from urine or blood) and other included metals

156 in logistic regression models was examined by the variance inflation factor (VIF), and

157 VIF value over 10 might indicate multicollinearity (Midi et al., 2010). To demonstrate

158 how interactions between metals might modify the magnitude of associations, a

159 sensitivity analysis was conducted to exclude other metals from urine or blood (single-

160 metal model).

161   We performed restricted cubic spline analysis with 3-knot (25th, 50th and 75th

162 percentiles) to detect the shape of dose-response relationships of urinary and blood Mn

163 levels with the odds of MetS and its components, using the median of urinary and blood

164 Mn as the reference point (Jin et al., 2018). We used the R *rms* package *anova* function

165 to estimate $P_{overall}$ and $P_{nonlinear}$, which indicated the statistical significance of dose-

166 response relationships. If $P_{overall}$ and $P_{non-linear}$ were less than 0.05, that indicated dose-

167 response relationship in non-linear manner. If only $P_{overall}$ was less than 0.05, that

168 indicated a dose-response relationship in linear manner.

169   We also built Bayesian kernel machine regression (BKMR) model (10,000

170 iterations for urinary and blood metals data) to evaluate the joint effects of urinary and

171 blood Mn and other included metals with the prevalence of MetS (Bobb et al., 2015).

172 We used posterior inclusion probabilities (PIP) to estimate the importance of Mn (from

173 urine or blood) and other metals in the association with MetS. A higher PIP indicates a

174 higher relative importance of a metal in the association with MetS when accounting for

175 the interactions with other included metals. All metals were log10-transformed for

176   BKMR analysis to reduce skewness. To further observe whether the urinary Mn-MetS

177   relationship varies substantially with the levels of included metals, exposure-outcome

178   function plots were made by fixing the other metals at their 25th, 50th or 75th percentile

179   levels. We also summarized the overall impact of the combined urinary metal mixture

180   on MetS in comparison to its 50th percentile.

181   To explore potential sex heterogeneities, we performed sex-stratified analysis in

182   the logistic regression models and added the interaction term between sex and Mn to

183   test for the significance of interactions. Given the inherent nature of multiple complex

184   survey designs, we accounted for sample weight for each participant in the NHANES

185   dataset. We used *svydesign* function in R to account for sampling weights, as well as

186   the stratification and clustering. Data was analyzed using R statistical software (Version

187   3.6.3). A two-sided *P* value of <0.05 was considered statistically significant.

188

189   **Results**

190   **Characteristics of the participants**

191   There were 1,713 participants (847 men and 866 women) and 3,335 participants

192   included in urinary and blood Mn analyses. Compared to women, fewer men received

193   college education. Men also had higher smoking rates, higher dietary energy intake,

194   higher rate of alcohol drinking and less prevalence in depression. There were significant

195   sex differences in each of the MetS component, men were more prevalent in elevated

196   FPG, TG, SBP and DBP, but less prevalent in elevated WC and low HDL-C than

197   women. The details of other demographic characteristics were described in **Table 1**.

Overall and sex-specific associations of urinary Mn with MetS

Among 15 urinary metals, the elastic net coefficients of seven metals (As, Cd, Hg, Ba, Mo, Sn and Ur) were not shrunken to zero by the elastic net penalty regression models, and therefore being included in the further logistic regression model to estimate single-metal exposure (Mn) with the odds of MetS after controlling for other metals (**Figure S2**).

When compared with Q1 of urinary Mn levels (**Table 2**), urinary Mn at Q3 was associated with a decreased odd of MetS among all participants (odds ratio [OR]=0.55, 95% confidence interval [C.I.] = 0.32-0.97) and men (OR= 0.40, 95% C.I.= 0.16-0.99). Urinary Mn at Q2 to Q4 had lower odds for elevated WC among all participants (Q4 versus Q1: OR=0.45, 95% C.I.= 0.25-0.80) and men (Q4 versus Q1: OR=0.29, 95% C.I.= 0.12-0.69), with significant decreased trends in odds ratio (P=0.031 for overall and P=0.017 for men). When treating urinary Mn as continuous variable (per log10 increment), urinary Mn inversely associated with the prevalence of elevated WC among all participants (OR= 0.50, 95% C.I.= 0.27-0.92) and men (OR= 0.35, 95% C.I. = 0.14-0.89). Compared with Q1, urinary Mn at Q3 also associated with a decreased odd of elevated FPG among all participants (OR= 0.46, 95% C.I. = 0.27-0.76) and men (OR= 0.44, 95% C.I. = 0.22-0.90). The interaction between sex and urinary Mn was not significant on the associations with MetS and its components. Interestingly, the per log10 increment of urinary Mn associated with an increased odd of elevated DBP among women (OR= 14.40, 95% C.I. =3.57-58.13). The wide 95% C.I. might be attributed by the lower rate of elevated DBP when compared to other MetS components,

especially when the results were stratified by sex. For the sensitivity analysis that added

creatinine as a separate covariate into regression models and used urinary Mn not

divided by creatinine as exposure, the per log10 increment of urinary Mn was

associated with the lower odd of MetS among men (OR= 0.14, 95% C.I. =0.03-0.61),

but not for women (OR= 2.26, 95% C.I. =0.62-8.28) and overall participants (OR= 1.08,

95% C.I. =0.41-2.83)

Overall and sex-specific associations of blood Mn with MetS

When compared with Q1 (**Table 3**), blood Mn at Q2 associated with a higher odd

of elevated WC (OR= 1.75, 95% C.I. = 1.05-2.92) among women, and blood Mn at Q4

associated with the higher odd of elevated FPG among women (OR= 2.01, 95% C.I. =

1.21-3.32). Per log10 increment of blood Mn associated with the decreased odds of

elevated WC among all participants (OR= 0.39, 95% C.I. = 0.21-0.74) and men (OR=

0.19, 95% C.I. = 0.08-0.48). The interaction between sex and blood Mn was not

significant on their associations with MetS and its components. Similar to urinary Mn,

the per log10 increment of blood Mn associated with the odd of elevated DBP among

women (OR= 11.01, 95% C.I. = 2.41-50.35), which might be attributed by the lower

rate of elevated DBP.

When looking into the collinearity issue of regression models between blood and

urinary metals with MetS and its components (**Table S1**), most VIF values were over

10 for urinary metals and all VIF values were under 10 for blood. The results indicated

that the problem of collinearity could be more apparent for urinary metals. Therefore,

we performed a sensitivity analysis to evaluate whether putting multiple metals into the

242 logistic regression model would affect the association between Mn (urine or blood) and

243 the odds of MetS. In the single-metal model, urinary Mn at Q3 was associated with a

244 lower odd of MetS among all participants (OR= 0.51, 95% C.I. = 0.30-0.85) and men

245 (OR= 0.36, 95% C.I. = 0.16-0.84), which was consistent to the results in multiple-metal

246 model (**Table S2**). Moreover, blood Mn did not have significant association with the

247 odd of MetS, which was also consistent to the results in multi-metal model (**Table S3**).

248 **Dose-response associations**

249   For dose-response relationship between urinary Mn and MetS, we observed a U-

250 shaped relationship among overall participants ($P_{overall}$ = 0.005, $P_{non-linear}$ = 0.008), an

251 inversed linear relationship among men ($P_{overall}$ = 0.005, $P_{non-linear}$ = 0.062), and no

252 significant association among women ($P_{overall}$ = 0.697, $P_{non-linear}$ = 0.486) (**Figure 1A**).

253 For blood Mn, we did not observe significant dose-response relationship among all

254 participants and both sexes (**Figure 1B**).

255   When looking into the components of MetS, urinary Mn had U-shaped relationship

256 with elevated WC ($P_{non-linear}$ = 0.028), low HDL-C ($P_{non-linear}$ = 0.003) and elevated FPG

257 ($P_{non-linear}$ = 0.009) among all participants (**Figure S3**). When stratified by sex, urinary

258 Mn had inverse relationship with elevated WC ($P_{overall}$ = 0.001, $P_{non-linear}$ = 0.281), low

259 HDL-C ($P_{overall}$ = 0.005, $P_{non-linear}$ = 0.054) and elevated FPG ($P_{overall}$ = 0.032, $P_{non-linear}$

260 = 0.096) among men (**Figure S4**), and elevated WC ($P_{overall}$ = 0.017, $P_{non-linear}$ = 0.616)

261 among women (**Figure S5**) in monotonic manner. Blood Mn was positively associated

262 with elevated FPG among all participants ($P_{overall}$ = 0.004, $P_{non-linear}$ = 0.053) (**Figure**

263 **S6**). Moreover, inverted U-shaped relationship was detected between blood Mn and

264   elevated FPG among men ($P_{\text{non-linear}}$ = 0.008) (**Figure S7**). Blood Mn positively

265   associated with elevated FPG (P_{overall} = 0.021, $P_{\text{non-linear}}$ = 0.482) among women (**Figure**

266   **S8**) in monotonic manner.

267   **BKMR analysis**

268   In the BKMR models, the relative importance of each metal on the odds of MetS

269   after accounting for inter-metal interaction was quantified by PIP (**Table S4**). For the

270   analysis of urinary metals, the PIP was 0.49 for Mn, 0.66 for Cd, 0.67 for Mo, 0.54 for

271   As, 0.91 for Hg, 0.58 for Ba, 0.55 for Sn, 0.59 for Ur. For the analysis of blood metals,

272   the PIP was 0.59 for Mn, 0.60 for Cd, Hg and Pb, and 0.61 for Se. As demonstrated in

273   the exposure-outcome function plot when fixing the other metals at their 25$^{\text{th}}$, 50$^{\text{th}}$ or

274   75$^{\text{th}}$ percentile levels (**Figure S9**), or fixing each of the included metal at their 10$^{\text{th}}$, 50$^{\text{th}}$

275   or 90$^{\text{th}}$ percentile levels (**Figure S10**), we did not observe the interactions of urinary

276   Mn and other metals with the odds of MetS. In the overall risk diagram, the combined

277   effect of urinary metal mixture has an inverse relationship with MetS (**Figure S11**).

278   **Discussion**

279   We have examined the associations of urinary and blood Mn with the odds of MetS

280   and its components among adults in NHANES 2011-2016. We have also explored the

281   potential sex-dependent heterogeneities. After adjusting for multiple covariates and the

282   levels of multiple metals in logistic regression models, the third quartile of urinary Mn

283   might associate with a lower odd of MetS, elevated WC and FPG among all participants

284   and men. The U-shaped dose-response relationship between urinary Mn and MetS

285   agrees with the results from regression analysis. Despite a larger sample size, we did

286   not observe statistically significant associations of blood Mn with the odds of MetS.

287   We have also compared the relative contributions of Mn and other metals on MetS using

288   BKMR analysis indicating that urinary Mn has less contribution in the odd of MetS

289   compared other metals, but the contribution of blood Mn was similar to other blood

290   metals.

291   Although not in a dose-response manner, our findings agree with previous studies

292   that demonstrated the protective effect of Mn against impaired glucose metabolism. For

293   instance, a cohort analysis of Women's Health Initiative (WHI) has shown that the

294   highest intake of dietary Mn has associated with 30% reduction in the risk of T2D

295   (Gong et al., 2020). The mediation analysis further suggested that the 19% and 12% of

296   T2D risk due to Mn were mediated through interleukin 6 and high-sensitivity C-reactive

297   protein respectively (Gong et al., 2020). The results from WHI have verified the roles

298   of Mn in cardiometabolic health through its anti-inflammatory function. Our research

299   group has also analyzed the associations of urinary Mn with fasting plasma glucose,

300   insulin resistance and kidney function among 1,417 participants in the NHANES (Yang

301   et al., 2020). In the previous analysis, urinary Mn has significant benefit in lowering

302   the level of fasting plasma glucose and raising the level of estimated glomerular

303   filtration rate, while blood Mn might associate with higher estimated glomerular

304   filtration rate among males. In the present analysis, we have expanded the analysis to

305   MetS and its components among NHANES participants.

306   In addition, we have increased the robustness of findings by accounting for the

307   interactions of urinary and blood metals (Domingo-Relloso et al., 2019). By

308 investigating the independent association between urinary and blood Mn and MetS, our

309 analysis may provide additional clinical value for to identify individuals with higher

310 odds of metabolic disorders. The use of elastic net analysis helped to identify metals

311 that may interact with Mn to influence cardiometabolic health, namely As, Cd, Hg, Ba,

312 Mo, Sn, Ur. From the BKMR results, we have quantified the relative importance of

313 each metal on the odds of MetS after accounting for inter-metal interactions and have

314 compared how Mn from urine and blood might have different contributions to the

315 prevalence of MetS. As demonstrated in PIP statistics, Mn from urine might not play

316 the most important role in the association between multiple metals exposure and the

317 odds of MetS given the lowest PIP amongst other urinary metals, although the PIP of

318 blood Mn (0.59) was comparable to other blood metals (0.60 to 0.61). This is an

319 interesting finding given the independent association between Mn and MetS after

320 adjusting for multiple confounders, which suggests the complexity in relationship that

321 researchers may not notice using conventional analysis plan. For other metals included

322 in this study, As, Cd and Hg may have dose-response toxicity to adverse

323 cardiometabolic health. (Roy et al., 2017; Noor et al., 2018; Spratlen et al., 2018).

324 However, Mn serves as both essential metals and neurotoxins depending on doses (Li

325 and Yang, 2018). The variation in the shape of relationship may weaken the overall

326 association with MetS. In addition, NHNAES was performed among general population

327 in U.S., the harmful effects of heavy metals might be less profound when comparing

328 with occupational workers.

329   On the other hand, our findings have suggested the differential associations of

330 urinary and blood Mn with the odds of MetS. While urinary Mn had significant inverse

331 associations with MetS and its components, the association with the odds of MetS was

332 attenuated for blood Mn. However, the association between urinary Mn and MetS may

333 be attributed by reverse causation. From the physiological perspective, the half-life of

334 blood Mn lasts for 10 to 42 days, but that for urinary Mn is less than 30 hours, indicating

335 a more recent exposure than blood Mn (Nelson et al., 1993). The lower level of urinary

336 Mn for people with MetS may reflect reduced urinary metal excretion due to impaired

337 renal function (Jin et al., 2018), a risk factor for adverse cardiometabolic health

338 (Sarafidis et al., 2006). The possibility of reverse causation is not ruled out given the

339 cross-sectional nature of the present study. Our hypothesis is also supported by the

340 overall risk diagram in BKMR analysis, which shows that the combined effect of

341 urinary metal mixture might have an inverse relationship with MetS.

342 Moreover, the present study has evaluated the sex differences in the relationship

343 between urinary Mn and MetS. For example, the third quartile of urinary Mn had an

344 inverse association with the MetS among men (OR at Q4: 0.40, 95% C.I. = 0.16-0.99)

345 but not women (OR= 0.63, 95% C.I. = 0.20-2.01). Sex differences in the response to

346 hormones may help to explain the relationship between Mn and cardiometabolic

347 biomarkers. Back in 2000s, researchers have found that higher testosterone levels and

348 lower plasma levels of sex hormone-binding globulin (SHBG) significantly linked to

349 an elevated risk of diabetes among women, and the relationship was weaker among

350 men (Ding et al., 2006; Ding et al., 2007). It is possible that Mn involves in the

351 interactions between SOD, SHBG and other sex hormones. However, correlation study

352   on the association between SOD and estradiol (a precursor of SHBG) may not fully

353   support this hypothesis (Skiljic et al., 2016), while low SHBG is associated with MetS

354   among men and women (Weinberg et al., 2006; Mohammed et al., 2018). Another

355   potential explanation is the sex difference in smoking habit. Previous study suggested

356   that smokers may have higher serum Mn levels than non-smokers due to exposure from

357   cigarettes (Ates Alkan et al., 2019). In our study sample, a higher proportion of males

358   were smokers, which may explain the sex-specific association in the odds of elevated

359   WC. However, we need to interpret the data cautiously because there is no significant

360   interaction between sex and Mn exposure in the logistic regression analysis. The

361   presence of sex-specific associations and the physiological mechanisms behind

362   warrants further investigation.

363   Besides, we have compared the magnitude of association and trend analysis from

364   logistic regression, with the dose-response relationship being tested from restricted

365   cubic spline analysis. From these comparisons, urinary Mn has demonstrated a non-

366   monotonic relationship with MetS (U-shaped association with MetS at Q2 and Q3 of

367   urinary Mn from logistic regression, $P_{\text{non-linear}} = 0.008$ in restricted cubic spline analysis)

368   among overall participants. While lower level of urinary Mn indicates the problem of

369   deficiency, diabetic patients with liver disorders may have increased excretion of Mn

370   from bile and urine (el-Yazigi et al., 1991). Moreover, blood Mn might have positive

371   association with elevated FPG among women (P trend=0.031 and positive association

372   at Q4 of blood Mn from logistic regression, $P_{\text{overall}} = 0.021$, $P_{\text{non-linear}} = 0.482$ in

373   restricted cubic spline analysis). As indicated by a review paper, blood Mn levels can

374   be increased or decreased among people with diabetes (Li and Yang, 2018). Although

375   the interpretation on possible sex-specific relationship should be cautious due to is no

376   significant interaction between sex and Mn exposure, our findings do provide additional

377   evidence into the body of knowledge.

378   The strength of the present study is the use of data collected by rigorous protocol

379   and extensive quality-control procedures, in which technicians were trained and

380   certified in data collection. However, we should interpret the study findings carefully

381   by noting several limitations. First, residual confounding effects could not be fully

382   addressed, such as the influence of Mn from dietary sources and physical activities on

383   cardiometabolic health. In addition, the cross-sectional nature of the present study was

384   not able to rule out how changes in metabolism, lifestyle or medication use may affect

385   the levels of urinary metal and cardiometabolic biomarkers. Another limitation is the

386   measurement of metal exposure at single time point, which may not reflect the long-

387   term and cumulative exposure. Moreover, findings from our study sample may not be

388   generalized to the total population in the U.S. despite accounting for the sampling

389   weight. Finally, Mn levels may vary by lifestyles and dietary patterns, therefore our

390   findings may not be directly extrapolated to all individuals.

391   To conclude, urinary Mn may associate with the odds of MetS with U-shaped

392   relationships and might have sex-specific differences. No significant associations were

393   observed between blood Mn and the odds of MetS. More mechanistic studies and

394   prospective cohorts are necessary to verify the potential sex and specimen specific roles

395   of Mn in cardiometabolic health.

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Manganese Exposure and Risk of Metabolic Syndrome (MetS)
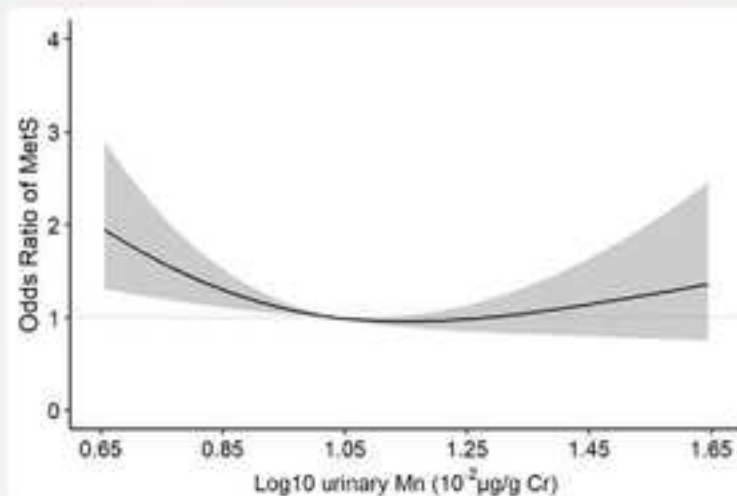
25

Mn

Manganese

54.938

Dose-response Relationships

Odds Ratio of MetS

Log10 urinary Mn (10^{-2} µg/g Cr)

Sex Differences

Components

↑ Triglyceride

↓ HDL-C

↑ Blood glucose

↑ Blood pressure

↑ Waist circumference

Highlights

- U-shaped relationship between urinary Mn and MetS was observed.

- No significant associations were found between blood Mn with MetS.

- There could be sex and specimen specific associations between Mn and MetS.

1   **Introduction**

2   Manganese (Mn) is an essential element in the human body that is mainly obtained

3   from water, nuts, grains, fruits, green vegetables, and caffeinated drinks (Gong et al.,

4   2020), which acts as a co-factor of superoxide dismutase (SOD), an enzyme responsible

5   for the degradation of superoxide radicals (Pfalzer and Bowman, 2017). Mn deficiency

6   may increase oxidative stress by producing more reactive oxygen species (ROS) (Li

7   and Yang, 2018), leading to inflammation and endothelial dysfunction (Liu et al., 2007;

8   Song et al., 2007), and accelerates the proliferation of vascular cells and increases

9   vasoconstriction (Tanito et al., 2004; Ward and Croft, 2006). Evidence from in vitro and

10   animal studies demonstrated that Mn supplementation could downregulate ROS

11   generation (Juttukonda et al., 2017), prevent endothelial dysfunction (Burlet and Jain,

12   2013) and reduce the levels of serum inflammatory biomarkers (Owumi and Dim, 2019).

13   However, excessive exposure of Mn from polluted air and water may lead to impaired

14   cognitive development and Parkinson's disease, especially among workers and general

15   populations residing near factories (Pfalzer and Bowman, 2017; Li and Yang, 2018).

16   Given the roles of Mn in alleviating oxidative stress, it may improve

17   cardiometabolic health with its anti-oxidative ability. Metabolic syndrome (MetS) is a

18   clustering of metabolic disorders, namely elevated waist circumference, impaired

19   glucose metabolism, elevated blood pressure and dyslipidemia (Expert Panel on

20   Detection, 2001), and is one of the precursors of various chronic diseases, including

21   diabetes, dementia, stroke and coronary heart disease (Pal et al., 2018; Saklayen, 2018).

22   When compared with other metal exposures, such as cadmium (Cd), lead (Pb), and

23   mercury (Hg) (Planchart et al., 2018; Ma et al., 2020), epidemiological evidence on the

24   relationship between Mn and MetS is limited and the results are inconsistent (Choi and

25   Bae, 2013; Li et al., 2013; Rhee et al., 2013; Rotter et al., 2015; Zhou et al., 2016; Bulka

26   et al., 2019). Most previous studies have used dietary methods to estimate Mn exposure.

27   In the analysis of Chinese National Nutrition and Health Survey 2010–2012 (Zhou et

28   al., 2016), dietary Mn was inversely associated with the odds of MetS, abdominal

29   obesity and elevated triglycerides among men, but positively associated with MetS and

30   low high-density-lipoprotein cholesterol (HDL-C) among women. In the analysis of the

31   Korea National Health and Nutrition Examination Survey (KNHANES) 2007–2008

32   (Choi and Bae, 2013), dietary Mn was lower among women with high blood pressure

33   but no significant difference among men. For another case-control study (Li et al., 2013),

34   dietary Mn was inversely associated with the odds of MetS and the number of MetS

35   components, but dietary assessment may subject to recall bias due to its self-reported

36   nature (Choi and Bae, 2013; Li et al., 2013; Zhou et al., 2016). Moreover, some studies

37   that have assessed Mn in urine or blood samples (Rhee et al., 2013; Rotter et al., 2015;

38   Bulka et al., 2019). A cross-sectional study in Poland found that serum Mn had

39   significant positive correlations with body mass index, waist circumference (WC),

40   waist-to-hip ratio, insulin, and insulin resistance, but no correlation with MetS (Rotter

41   et al., 2015). In analysis of KNHANES 2008 (Rhee et al., 2013), Mn from whole blood

42   or urine did not have significant relationship with MetS. A recent cross-sectional

43   analysis of the United States (U.S.) NHANES 2011–2014 did not find significant

44   association between Mn from whole blood with the prevalence of MetS, nor for the

45 methylmercury-manganese pattern using principal components analysis (Bulka et al.,

46 2019). Furthermore, our previous analysis among the U.S. NHANES participants has

47 found positive linear relationships between urinary Mn with fasting plasma glucose

48 among women, and J-shaped dose-response relationships of blood Mn with insulin

49 resistance among men (Yang et al., 2020). Although some analyses explored the

50 combined effects of metal including Mn on cardiometabolic health, there is a need to

51 investigate associations of Mn with MetS in a sex-specific manner.

52 To address the knowledge gap as mentioned above, we have analyzed the

53 NHANES data from a nationwide sample in the U.S. to investigate the overall and sex-

54 specific associations of urinary and blood Mn levels with the prevalence of MetS and

55 its components. We also explored the shape of dose-response relationships across the

56 doses of urinary and blood Mn levels and the prevalence of MetS.

57

58 **Materials and methods**

59 **Study population**

60 The U.S. NHANES program refers to multiple cross-sectional surveys conducted

61 among general population in the U.S. to collect data on diet, nutritional status, health

62 status and health behaviors of the participants. In the present study, we included data (n

63 =29,902) from surveys conducted in 2011-2012, 2013-2014, and 2015-2016 cycles. A

64 random subsample was performed for urinary and blood Mn, and other metal assays

65 during the three cycles (Centers for Disease Control and Prevention, 2011). We

66   included participants separately for analysis, the only difference in inclusion criteria

67   was the data availability of metals from urine or whole blood. For analyzing the

68   relationship between urinary Mn and MetS, we excluded participants who were missing

69   urinary metals data (n = 21,707), with missing value in biomarkers related to MetS (n

70   = 5,539), aged <18 years (n = 385), and with gestational diabetes mellitus (n = 1) or

71   gestational hypertension (n = 2). Since cardiovascular disease might influence the

72   association of urinary Mn levels with MetS, we further excluded participants with type

73   2 diabetes (T2D), coronary heart disease and stroke based on self-reported medical

74   history (n=556). Finally, 1,713 participants were included in urinary Mn analyses

75   (**Figure S1**). For analyzing the relationship between blood Mn and MetS, we applied

76   the same exclusion criteria and included 3,335 participants in the final analyses (**Figure**

77   **S1**).

78   **Measurements of Mn and other metals**

79   With the use of inductively coupled plasma mass spectrometers (ELAN® 6100

80   DRC^{Plus} or ELAN® DRC II, PerkinElmer Norwalk, Fairfield), 15 metal levels in urine,

81   namely Cobalt (Co), Mn, Cd, Hg, Arsenic (As), Pb, Molybdenum (Mo), Barium (Ba),

82   Cesium (Cs), Antimony (Sb), Tin (Sn), Strontium (Sr), Thallium (TL), Tungsten (W)

83   and Uranium (U), along with 5 metal levels in whole blood, namely Cd, Hg, Pb,

84   Selenium (Se) and Mn were measured. Spot urine and fasting blood sample were

85   collected at the time of the laboratory exam, shipped on dry ice to the National Center

86   for Environmental Health (NCEH) in Atlanta, GA, and stored frozen at −20 °C until

87   assayed (Centers for Disease Control and Prevention, 2011).

88   For urinary and blood metals that had levels below the detection limit, the values

89   were substituted with the detection limit divided by the square root of two. If the

90   detection limit differed between the three survey cycles, we selected the highest value

91   of the three cycles. The NHANES quality assurance and quality control (QA/QC)

92   protocols have met the 1988 Clinical Laboratory Improvement Act mandates (Bachner

93   and Hamlin, 1993a, b).

94   **MetS Definition**

95   MetS was defined according to the diagnostic criteria proposed by the Adult

96   Treatment Program III of the National Cholesterol Education Program (Expert Panel

97   on Detection, 2001). Participants with three or more of the following conditions were

98   classified as having MetS: 1) elevated triglyceride (TG) $\geq$ 1.69 mmol/L (150 mg/dL);

99   2) low HDL-C : men < 1.04 mmol/L (40 mg/dL), women < 1.29 mmol/L (50 mg/dL);

100 3) elevated fasting plasma glucose (FPG): $\geq$6.1 mmol/L (110 mg/dL); 4) elevated WC:

101 men $\geq$ 102 cm, women $\geq$ 88 cm; 5) elevated systolic blood pressure (SBP) $\geq$ 130 mmHg

102 and/or elevated diastolic blood pressure (DBP) $\geq$ 85 mmHg. Fasting blood samples

103 were examined in the morning session after a 9-hour fast, while blood pressure was

104 measured by physicians for three times to calculate the average value (Centers for

105 Disease Control and Prevention, 2011).

106 **Covariates**

107 Standardized questionnaires were used to obtain socio-demographics, lifestyle,

108 clinical, and nutritional factors from participants. We included covariates based on the

109 priori knowledge on the risk factors of adverse cardiometabolic health, including low

110   socioeconomic status (Ross et al., 2020), severity of depression (Godin et al., 2019),

111   smoking status (Adisen et al., 2018), excessive daily energy intake (Xu et al., 2018),

112   alcohol drinking status (Adisen et al., 2018), and the history of cancer (Bishehsari et al.,

113   2020). We included age (18-39, 40-59 or $\geq$60 years), sex (men or women),

114   race/ethnicity (Non-Hispanic White, Non-Hispanic Black, other Hispanic or other race),

115   educational attainment (less than high school, high school, at least some college),

116   poverty income ratio (PIR, dichotomized as <1 or $\geq$1), smoking status (categorized as

117   never, former or current smoker), dietary energy intake in quartiles, status of alcohol

118   drinking (dichotomized as yes or no), depression as classified by Patient Health

119   Questionnaire-9 (categorized as no depression, mild depression, moderate to severe

120   depression) (Patel et al., 2019), and self-reported history of cancer. Poverty-income

121   ratio (PIR) is the ratio of the family's self-reported income to the family's appropriate

122   poverty threshold according to the U.S. Census Bureau, and PIR values of 1.00 or

123   greater indicating people above the poverty threshold (Tyrrell et al., 2013). To calculate

124   daily energy intake, participants reported the food and beverage items reported

125   consumed between midnight and midnight 24 hours prior to the NHANES dietary

126   interview (Marriott et al., 2019).

127   **Statistical analysis**

128   Descriptive statistics were used to present the frequency and proportion of the

129   demographic. We compared the sex differences in participants' characteristics by chi-

130   square test. Urinary Mn was divided by urinary creatinine to control the concentration

131   dilution of urine. To account for the collinear nature of 15 urinary metals being

132 measured, we firstly conducted elastic net penalty regression to select the potential

133 metals that associated with MetS (Stafoggia et al., 2017; Hou et al., 2019). The elastic

134 net model can perform selection while enabling the inclusion of collinear predictors

135 through combining the least absolute shrinkage and selection operator and ridge (Zhao

136 et al., 2020). A set of elastic net coefficients were estimated and selected the metals that

137 their elastic net coefficients were not shrunken to zero. (Zhao et al., 2020)

138   Urinary and blood Mn levels was treated as categorical variable (classified as

139 quartiles, Q1 to Q4) or continuous variable (log10-transformed to reduce skewness).

140 Logistic regression models were used to examine the associations between urinary Mn

141 levels and the odds of MetS and each of its components (elevated TG, low HDL-C,

142 elevated FPG, elevated WC, elevated SBP and elevated DBP). The regression models

143 were adjusted for age, sex, race, education attainment, poverty income ratio, smoking

144 status, status of alcohol drinking, dietary energy, severity of depression, self-reported

145 history of cancer and urinary metals (all categorized in quartiles) as selected by elastic

146 net regression (multi-metal model). Tests of linear trend across increasing quartiles (Q)

147 of urinary Mn levels were performed by assigning the median levels in quartiles and

148 being treated as a continuous variable. Sensitivity analysis was performed to examine

149 the relationship between urinary Mn and MetS. We have added creatinine as a separate

150 covariate into regression models and used urinary Mn as exposure without dividing it

151 by creatinine (Barr et al., 2005).

152   The analysis plan when using blood Mn as exposure was the same as urinary Mn

153 except for not including urinary creatinine, with all blood metals, namely Cd, Hg, Pb,

154 Se (all categorized in quartiles) being included into the logistic regression model (multi-

155 metal model). Collinearity between Mn (from urine or blood) and other included metals

156 in logistic regression models was examined by the variance inflation factor (VIF), and

157 VIF value over 10 might indicate multicollinearity (Midi et al., 2010). To demonstrate

158 how interactions between metals might modify the magnitude of associations, a

159 sensitivity analysis was conducted to exclude other metals from urine or blood (single-

160 metal model).

161 We performed restricted cubic spline analysis with 3-knot (25th, 50th and 75th

162 percentiles) to detect the shape of dose-response relationships of urinary and blood Mn

163 levels with the odds of MetS and its components, using the median of urinary and blood

164 Mn as the reference point (Jin et al., 2018). We used the R *rms* package *anova* function

165 to estimate $P_{overall}$ and $P_{nonlinear}$, which indicated the statistical significance of dose-

166 response relationships. If $P_{overall}$ and $P_{non-linear}$ were less than 0.05, that indicated dose-

167 response relationship in non-linear manner. If only $P_{overall}$ was less than 0.05, that

168 indicated a dose-response relationship in linear manner.

169 We also built Bayesian kernel machine regression (BKMR) model (10,000

170 iterations for urinary and blood metals data) to evaluate the joint effects of urinary and

171 blood Mn and other included metals with the prevalence of MetS (Bobb et al., 2015).

172 We used posterior inclusion probabilities (PIP) to estimate the importance of Mn (from

173 urine or blood) and other metals in the association with MetS. A higher PIP indicates a

174 higher relative importance of a metal in the association with MetS when accounting for

175 the interactions with other included metals. All metals were log10-transformed for

176 BKMR analysis to reduce skewness. To further observe whether the urinary Mn-MetS

177 relationship varies substantially with the levels of included metals, exposure-outcome

178 function plots were made by fixing the other metals at their 25th, 50th or 75th percentile

179 levels. We also summarized the overall impact of the combined urinary metal mixture

180 on MetS in comparison to its 50th percentile.

181 To explore potential sex heterogeneities, we performed sex-stratified analysis in

182 the logistic regression models and added the interaction term between sex and Mn to

183 test for the significance of interactions. Given the inherent nature of multiple complex

184 survey designs, we accounted for sample weight for each participant in the NHANES

185 dataset. We used *svydesign* function in R to account for sampling weights, as well as

186 the stratification and clustering. Data was analyzed using R statistical software (Version

187 3.6.3). A two-sided *P* value of <0.05 was considered statistically significant.

188

189 **Results**

190 **Characteristics of the participants**

191 There were 1,713 participants (847 men and 866 women) and 3,335 participants

192 included in urinary and blood Mn analyses. Compared to women, fewer men received

193 college education. Men also had higher smoking rates, higher dietary energy intake,

194 higher rate of alcohol drinking and less prevalence in depression. There were significant

195 sex differences in each of the MetS component, men were more prevalent in elevated

196 FPG, TG, SBP and DBP, but less prevalent in elevated WC and low HDL-C than

197 women. The details of other demographic characteristics were described in **Table 1**.

Overall and sex-specific associations of urinary Mn with MetS

198

Among 15 urinary metals, the elastic net coefficients of seven metals (As, Cd, Hg,

199

Ba, Mo, Sn and Ur) were not shrunken to zero by the elastic net penalty regression

200

models, and therefore being included in the further logistic regression model to estimate

201

single-metal exposure (Mn) with the odds of MetS after controlling for other metals

202

(**Figure S2**).

203

When compared with Q1 of urinary Mn levels (**Table 2**), urinary Mn at Q3 was

204

associated with a decreased odd of MetS among all participants (odds ratio [OR]=0.55,

205

95% confidence interval [C.I.] = 0.32-0.97) and men (OR= 0.40, 95% C.I.= 0.16-0.99).

206

Urinary Mn at Q2 to Q4 had lower odds for elevated WC among all participants (Q4

207

versus Q1: OR=0.45, 95% C.I.= 0.25-0.80) and men (Q4 versus Q1: OR=0.29, 95%

208

C.I.= 0.12-0.69), with significant decreased trends in odds ratio (*P*=0.031 for overall

209

and *P*=0.017 for men). When treating urinary Mn as continuous variable (per log10

210

increment), urinary Mn inversely associated with the prevalence of elevated WC among

211

all participants (OR= 0.50, 95% C.I.= 0.27-0.92) and men (OR= 0.35, 95% C.I. = 0.14-

212

0.89). Compared with Q1, urinary Mn at Q3 also associated with a decreased odd of

213

elevated FPG among all participants (OR= 0.46, 95% C.I. = 0.27-0.76) and men (OR=

214

0.44, 95% C.I. = 0.22-0.90). The interaction between sex and urinary Mn was not

215

significant on the associations with MetS and its components. Interestingly, the per

216

log10 increment of urinary Mn associated with an increased odd of elevated DBP

217

among women (OR= 14.40, 95% C.I. =3.57-58.13). The wide 95% C.I. might be

218

attributed by the lower rate of elevated DBP when compared to other MetS components,

219

220 especially when the results were stratified by sex. For the sensitivity analysis that added

221 creatinine as a separate covariate into regression models and used urinary Mn not

222 divided by creatinine as exposure, the per log10 increment of urinary Mn was

223 associated with the lower odd of MetS among men (OR= 0.14, 95% C.I. =0.03-0.61),

224 but not for women (OR= 2.26, 95% C.I. =0.62-8.28) and overall participants (OR= 1.08,

225 95% C.I. =0.41-2.83)

Overall and sex-specific associations of blood Mn with MetS

227 When compared with Q1 (**Table 3**), blood Mn at Q2 associated with a higher odd

228 of elevated WC (OR= 1.75, 95% C.I. = 1.05-2.92) among women, and blood Mn at Q4

229 associated with the higher odd of elevated FPG among women (OR= 2.01, 95% C.I. =

230 1.21-3.32). Per log10 increment of blood Mn associated with the decreased odds of

231 elevated WC among all participants (OR= 0.39, 95% C.I. = 0.21-0.74) and men (OR=

232 0.19, 95% C.I. = 0.08-0.48). The interaction between sex and blood Mn was not

233 significant on their associations with MetS and its components. Similar to urinary Mn,

234 the per log10 increment of blood Mn associated with the odd of elevated DBP among

235 women (OR= 11.01, 95% C.I. = 2.41-50.35), which might be attributed by the lower

236 rate of elevated DBP.

237 When looking into the collinearity issue of regression models between blood and

238 urinary metals with MetS and its components (**Table S1**), most VIF values were over

239 10 for urinary metals and all VIF values were under 10 for blood. The results indicated

240 that the problem of collinearity could be more apparent for urinary metals. Therefore,

241 we performed a sensitivity analysis to evaluate whether putting multiple metals into the

242   logistic regression model would affect the association between Mn (urine or blood) and

243   the odds of MetS. In the single-metal model, urinary Mn at Q3 was associated with a

244   lower odd of MetS among all participants (OR= 0.51, 95% C.I. = 0.30-0.85) and men

245   (OR= 0.36, 95% C.I. = 0.16-0.84), which was consistent to the results in multiple-metal

246   model (**Table S2**). Moreover, blood Mn did not have significant association with the

247   odd of MetS, which was also consistent to the results in multi-metal model (**Table S3**).

248   **Dose-response associations**

249   For dose-response relationship between urinary Mn and MetS, we observed a U-

250   shaped relationship among overall participants ($P_{overall} = 0.005$, $P_{non-linear} = 0.008$), an

251   inversed linear relationship among men ($P_{overall} = 0.005$, $P_{non-linear} = 0.062$), and no

252   significant association among women ($P_{overall} = 0.697$, $P_{non-linear} = 0.486$) (**Figure 1A**).

253   For blood Mn, we did not observe significant dose-response relationship among all

254   participants and both sexes (**Figure 1B**).

255   When looking into the components of MetS, urinary Mn had U-shaped relationship

256   with elevated WC ($P_{non-linear} = 0.028$), low HDL-C ($P_{non-linear} = 0.003$) and elevated FPG

257   ($P_{non-linear} = 0.009$) among all participants (**Figure S3**). When stratified by sex, urinary

258   Mn had inverse relationship with elevated WC ($P_{overall} = 0.001$, $P_{non-linear} = 0.281$), low

259   HDL-C ($P_{overall} = 0.005$, $P_{non-linear} = 0.054$) and elevated FPG ($P_{overall} = 0.032$, $P_{non-linear}$

260    $= 0.096$) among men (**Figure S4**), and elevated WC ($P_{overall} = 0.017$, $P_{non-linear} = 0.616$)

261   among women (**Figure S5**) in monotonic manner. Blood Mn was positively associated

262   with elevated FPG among all participants ($P_{overall} = 0.004$, $P_{non-linear} = 0.053$) (**Figure**

263   **S6**). Moreover, inverted U-shaped relationship was detected between blood Mn and

264 elevated FPG among men ($P_{\text{non-linear}} = 0.008$) (**Figure S7**). Blood Mn positively

265 associated with elevated FPG ($P_{\text{overall}} = 0.021$, $P_{\text{non-linear}} = 0.482$) among women (**Figure**

266 **S8**) in monotonic manner.

BKMR analysis

268   In the BKMR models, the relative importance of each metal on the odds of MetS

269 after accounting for inter-metal interaction was quantified by PIP (**Table S4**). For the

270 analysis of urinary metals, the PIP was 0.49 for Mn, 0.66 for Cd, 0.67 for Mo, 0.54 for

271 As, 0.91 for Hg, 0.58 for Ba, 0.55 for Sn, 0.59 for Ur. For the analysis of blood metals,

272 the PIP was 0.59 for Mn, 0.60 for Cd, Hg and Pb, and 0.61 for Se. As demonstrated in

273 the exposure-outcome function plot when fixing the other metals at their 25th, 50th or

274 75th percentile levels (**Figure S9**), or fixing each of the included metal at their 10th, 50th

275 or 90th percentile levels (**Figure S10**), we did not observe the interactions of urinary

276 Mn and other metals with the odds of MetS. In the overall risk diagram, the combined

277 effect of urinary metal mixture has an inverse relationship with MetS (**Figure S11**).

Discussion

279   We have examined the associations of urinary and blood Mn with the odds of MetS

280 and its components among adults in NHANES 2011-2016. We have also explored the

281 potential sex-dependent heterogeneities. After adjusting for multiple covariates and the

282 levels of multiple metals in logistic regression models, the third quartile of urinary Mn

283 might associate with a lower odd of MetS, elevated WC and FPG among all participants

284 and men. The U-shaped dose-response relationship between urinary Mn and MetS

285 agrees with the results from regression analysis. Despite a larger sample size, we did

not observe statistically significant associations of blood Mn with the odds of MetS.

We have also compared the relative contributions of Mn and other metals on MetS using

BKMR analysis indicating that urinary Mn has less contribution in the odd of MetS

compared other metals, but the contribution of blood Mn was similar to other blood

metals.

Although not in a dose-response manner, our findings agree with previous studies

that demonstrated the protective effect of Mn against impaired glucose metabolism. For

instance, a cohort analysis of Women's Health Initiative (WHI) has shown that the

highest intake of dietary Mn has associated with 30% reduction in the risk of T2D

(Gong et al., 2020). The mediation analysis further suggested that the 19% and 12% of

T2D risk due to Mn were mediated through interleukin 6 and high-sensitivity C-reactive

protein respectively (Gong et al., 2020). The results from WHI have verified the roles

of Mn in cardiometabolic health through its anti-inflammatory function. Our research

group has also analyzed the associations of urinary Mn with fasting plasma glucose,

insulin resistance and kidney function among 1,417 participants in the NHANES (Yang

et al., 2020). In the previous analysis, urinary Mn has significant benefit in lowering

the level of fasting plasma glucose and raising the level of estimated glomerular

filtration rate, while blood Mn might associate with higher estimated glomerular

filtration rate among males. In the present analysis, we have expanded the analysis to

MetS and its components among NHANES participants.

In addition, we have increased the robustness of findings by accounting for the

interactions of urinary and blood metals (Domingo-Relloso et al., 2019). By

308   investigating the independent association between urinary and blood Mn and MetS, our

309   analysis may provide additional clinical value for to identify individuals with higher

310   odds of metabolic disorders. The use of elastic net analysis helped to identify metals

311   that may interact with Mn to influence cardiometabolic health, namely As, Cd, Hg, Ba,

312   Mo, Sn, Ur. From the BKMR results, we have quantified the relative importance of

313   each metal on the odds of MetS after accounting for inter-metal interactions and have

314   compared how Mn from urine and blood might have different contributions to the

315   prevalence of MetS. As demonstrated in PIP statistics, Mn from urine might not play

316   the most important role in the association between multiple metals exposure and the

317   odds of MetS given the lowest PIP amongst other urinary metals, although the PIP of

318   blood Mn (0.59) was comparable to other blood metals (0.60 to 0.61). This is an

319   interesting finding given the independent association between Mn and MetS after

320   adjusting for multiple confounders, which suggests the complexity in relationship that

321   researchers may not notice using conventional analysis plan. For other metals included

322   in this study, As, Cd and Hg may have dose-response toxicity to adverse

323   cardiometabolic health. (Roy et al., 2017; Noor et al., 2018; Spratlen et al., 2018).

324   However, Mn serves as both essential metals and neurotoxins depending on doses (Li

325   and Yang, 2018). The variation in the shape of relationship may weaken the overall

326   association with MetS. In addition, NHNAES was performed among general population

327   in U.S., the harmful effects of heavy metals might be less profound when comparing

328   with occupational workers.

329   On the other hand, our findings have suggested the differential associations of

330   urinary and blood Mn with the odds of MetS. While urinary Mn had significant inverse

331   associations with MetS and its components, the association with the odds of MetS was

332   attenuated for blood Mn. However, the association between urinary Mn and MetS may

333   be attributed by reverse causation. From the physiological perspective, the half-life of

334   blood Mn lasts for 10 to 42 days, but that for urinary Mn is less than 30 hours, indicating

335   a more recent exposure than blood Mn (Nelson et al., 1993). The lower level of urinary

336   Mn for people with MetS may reflect reduced urinary metal excretion due to impaired

337   renal function (Jin et al., 2018), a risk factor for adverse cardiometabolic health

338   (Sarafidis et al., 2006). The possibility of reverse causation is not ruled out given the

339   cross-sectional nature of the present study. Our hypothesis is also supported by the

340   overall risk diagram in BKMR analysis, which shows that the combined effect of

341   urinary metal mixture might have an inverse relationship with MetS.

342   Moreover, the present study has evaluated the sex differences in the relationship

343   between urinary Mn and MetS. For example, the third quartile of urinary Mn had an

344   inverse association with the MetS among men (OR at Q4: 0.40, 95% C.I. = 0.16-0.99)

345   but not women (OR= 0.63, 95% C.I. = 0.20-2.01). Sex differences in the response to

346   hormones may help to explain the relationship between Mn and cardiometabolic

347   biomarkers. Back in 2000s, researchers have found that higher testosterone levels and

348   lower plasma levels of sex hormone-binding globulin (SHBG) significantly linked to

349   an elevated risk of diabetes among women, and the relationship was weaker among

350   men (Ding et al., 2006; Ding et al., 2007). It is possible that Mn involves in the

351   interactions between SOD, SHBG and other sex hormones. However, correlation study

352   on the association between SOD and estradiol (a precursor of SHBG) may not fully

353   support this hypothesis (Skiljic et al., 2016), while low SHBG is associated with MetS

354   among men and women (Weinberg et al., 2006; Mohammed et al., 2018). Another

355   potential explanation is the sex difference in smoking habit. Previous study suggested

356   that smokers may have higher serum Mn levels than non-smokers due to exposure from

357   cigarettes (Ates Alkan et al., 2019). In our study sample, a higher proportion of males

358   were smokers, which may explain the sex-specific association in the odds of elevated

359   WC. However, we need to interpret the data cautiously because there is no significant

360   interaction between sex and Mn exposure in the logistic regression analysis. The

361   presence of sex-specific associations and the physiological mechanisms behind

362   warrants further investigation.

363   Besides, we have compared the magnitude of association and trend analysis from

364   logistic regression, with the dose-response relationship being tested from restricted

365   cubic spline analysis. From these comparisons, urinary Mn has demonstrated a non-

366   monotonic relationship with MetS (U-shaped association with MetS at Q2 and Q3 of

367   urinary Mn from logistic regression, $P_{\text{non-linear}} = 0.008$ in restricted cubic spline analysis)

368   among overall participants. While lower level of urinary Mn indicates the problem of

369   deficiency, diabetic patients with liver disorders may have increased excretion of Mn

370   from bile and urine (el-Yazigi et al., 1991). Moreover, blood Mn might have positive

371   association with elevated FPG among women (P trend=0.031 and positive association

372   at Q4 of blood Mn from logistic regression, $P_{\text{overall}} = 0.021$, $P_{\text{non-linear}} = 0.482$ in

373   restricted cubic spline analysis). As indicated by a review paper, blood Mn levels can

be increased or decreased among people with diabetes (Li and Yang, 2018). Although the interpretation on possible sex-specific relationship should be cautious due to is no significant interaction between sex and Mn exposure, our findings do provide additional evidence into the body of knowledge.

The strength of the present study is the use of data collected by rigorous protocol and extensive quality-control procedures, in which technicians were trained and certified in data collection. However, we should interpret the study findings carefully by noting several limitations. First, residual confounding effects could not be fully addressed, such as the influence of Mn from dietary sources and physical activities on cardiometabolic health. In addition, the cross-sectional nature of the present study was not able to rule out how changes in metabolism, lifestyle or medication use may affect the levels of urinary metal and cardiometabolic biomarkers. Another limitation is the measurement of metal exposure at single time point, which may not reflect the long-term and cumulative exposure. Moreover, findings from our study sample may not be generalized to the total population in the U.S. despite accounting for the sampling weight. Finally, Mn levels may vary by lifestyles and dietary patterns, therefore our findings may not be directly extrapolated to all individuals.

To conclude, urinary Mn may associate with the odds of MetS with U-shaped relationships and might have sex-specific differences. No significant associations were observed between blood Mn and the odds of MetS. More mechanistic studies and prospective cohorts are necessary to verify the potential sex and specimen specific roles of Mn in cardiometabolic health.

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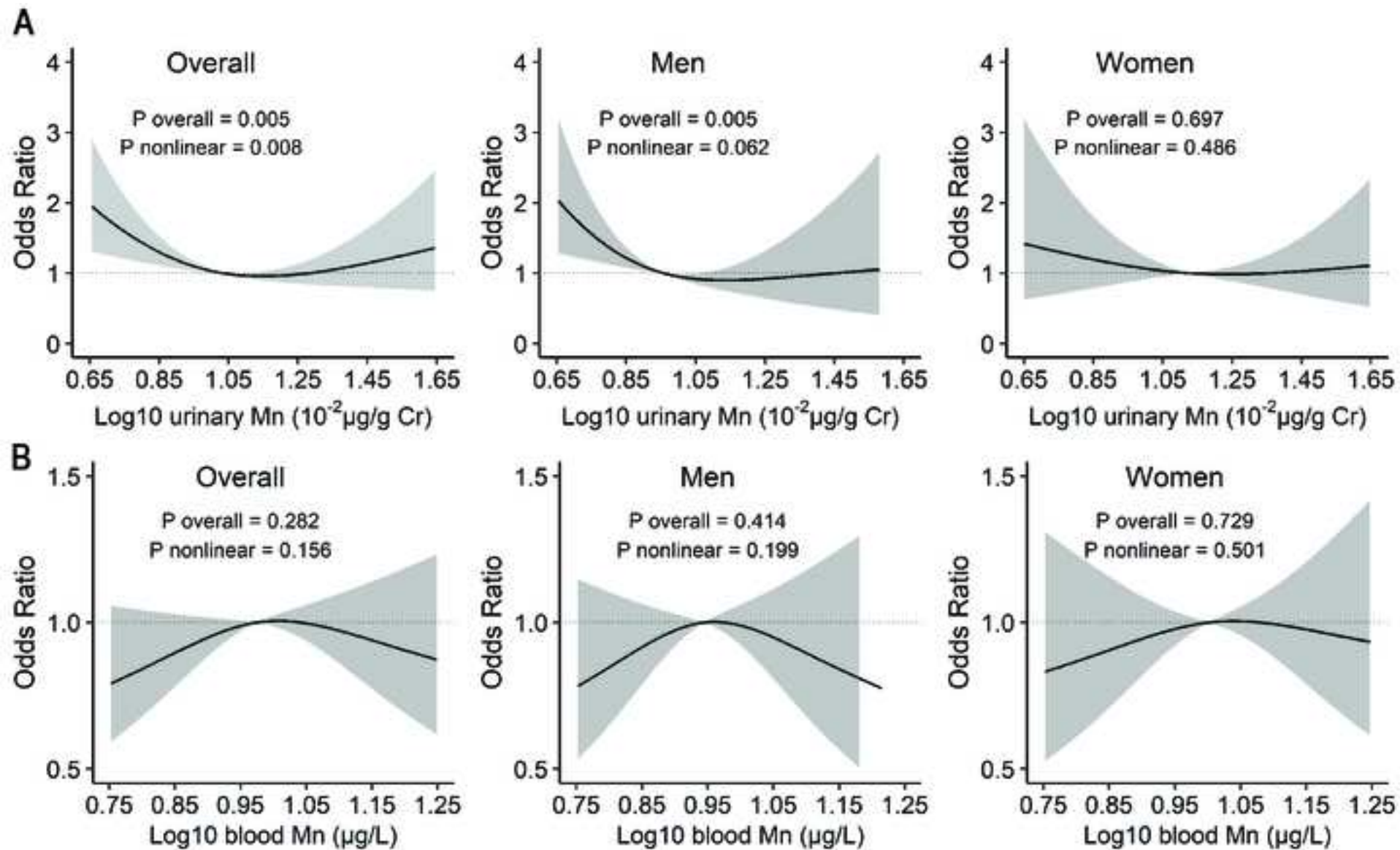
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Figure 1. Restricted cubic spline analysis of dose-response relationships between urinary (A) and blood (B) manganese (Mn) and metabolic syndrome.

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CRediT authorship contribution statement

Kenneth Lo: Conceptualization, Writing - original draft, Writing - review & editing, **Jing-Li Yang:** Conceptualization, Formal analysis, Writing - review & editing, Visualization, **Ai-min Yang:** Conceptualization, Writing - review & editing, Visualization, Supervision, **Chao-Lei Chen:** Writing - review & editing, **Lin Liu:** Writing - review & editing, **Yu-Qing Huang:** Writing - review & editing, **Ying-Qing Feng:** Conceptualization, Writing - review & editing, Supervision. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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.