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Sex-specific and dose-response relationships of urinary cobalt and molybdenum levels with glucose levels and insulin resistance in U.S. adults

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ABSTRACT

Growing studies have linked metal exposure to diabetes risk. However, these studies had inconsistent results. We used a multiple linear regression model to investigate the sex-specific and dose-response associations between urinary metals (cobalt (Co) and molybdenum (Mo)) and diabetes-related indicators (fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), homeostasis model assessment for insulin resistance (HOMA-IR), and insulin) in a cross-sectional study based on the United States National Health and Nutrition Examination Survey. The urinary metal concentrations of 1423 eligible individuals were stratified on the basis of the quartile distribution. Our results showed that the urinary Co level in males at the fourth quartile (Q4) was strongly correlated with increased FPG ($\beta = 0.61$, 95% CI: 0.17–1.04), HbA1c ($\beta = 0.31$, 95% CI: 0.09–0.54), insulin ($\beta = 8.18$, 95% CI: 2.84–13.52), and HOMA–IR ($\beta = 3.42$, 95% CI: 1.40–5.44) when compared with first quartile (Q1). High urinary Mo levels (Q4 vs. Q1) were associated with elevated FPG ($\beta = 0.46$, 95% CI: 0.17–0.75) and HbA1c ($\beta = 0.27$, 95% CI: 0.11–0.42) in the overall population. Positive linear dose-response associations were observed between urinary Co and insulin ($P_{nonlinear} = 0.513$) and HOMA–IR ($P_{nonlinear} = 0.736$) in males, as well as a positive linear dose-response relationship between urinary Mo and FPG ($P_{nonlinear} = 0.826$) and HbA1c ($P_{nonlinear} = 0.376$) in the overall population. Significant sex-specific and dose-response relationships were observed between urinary metals (Co and Mo) and diabetes-related indicators, and the potential mechanisms should be further investigated.

Introduction

Diabetes, which is characterized by abnormalities in insulin secretion and/or function, has become a common chronic noncommunicable disease and a major threat to human health worldwide (ADA, 2019). According to the International Diabetes Federation (IDF), the global prevalence of diabetes in 2019 is 9.3% with 463 million people impacted (IDF, 2019). However, the prevalence of diabetes has been steadily growing for decades worldwide. Estimated by the IDF, the number of diabetes patients is expected to reach 599 million (global

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prevalence of 10.2%) by 2030 and 700 million (10.9%) by 2045 (IDF, 2019). Hemoglobin A1c (HbA1c) is the combination of red blood cell hemoglobin and serum sugar that may accurately indicate the average level of plasma glycemia in diabetes individuals during the last 2–3 months (Hotta et al., 2018). The homeostasis model assessment for insulin resistance (HOMA-IR) is widely acceptable for detecting and assessing the severity of insulin resistance (Wang et al., 2020b). In general, HbA1c, fasting plasma glucose (FPG), insulin, and HOMA-IR are generally regarded as the primary biomarkers during the onset and progression of diabetes. Early diagnosis and management of these markers can significantly benefit the high-risk group by decreasing diabetes risk and improving prognosis.

In recent years, the impact of environmental pollution on health has attracted considerable attention. Pilot studies have indicated the importance of metal exposure on the progression of diabetes (Kuo et al., 2013; Wang et al., 2020a). Epidemiological studies have demonstrated that molybdenum (Mo) and cobalt (Co) exposure may be associated with the increased risk of diabetes (Liu et al., 2016; Menke et al., 2016). However, few studies have focused on the associations between Co and Mo exposure and FPG, HbA1c, insulin, and HOMA-IR (Hansen et al., 2017; Liu et al., 2016; Menke et al., 2016; Xiao et al., 2018). The absence of the evaluation of multiple-metal exposure may further bias the current understanding of the health effect of environmental metal exposures. It should be noted that metal exposures are often mixed, that existing techniques may be hampered by multicollinearity and model misspecification.

Therefore, a recently proposed statistical method (the Bayesian kernel machine regression model, BKMR) (Bobb et al., 2015) was used in our study to evaluate the associations between urinary Mo and Co concentrations and diabetes-related biomarkers, as well as to explore the health effects of multiple-metal exposure. In addition, studies have suggested that the health impacts of metal exposure linked to diabetes may have a strong gender-specific effects (Yang et al., 2020; Zheng et al., 2018). Therefore, the sex-specific effects should also be considered in further analysis.

In this study, we aimed to explore (1) the associations between urinary Co and Mo concentrations and diabetes-related biomarkers, (2) the potential sex-specific and dose-response associations, and (3) interaction of urinary metals among adults in the U.S. National Health and Nutrition Examination Survey (NHANES) from 2011 to 2016.

1. Methods

1.1. Study population

The participants of our study were selected from the U.S. NHANES database as described previously (Zipf et al., 2013). In brief, the U.S. NHANES was a series of representative national health and nutrition examination surveys in the United States, which was conducted every 2 years. All participants provided a written informed consent prior to their inclusion in the study. Data from adjacent survey cycles can be combined to analyze based on the NHANES analytic guidelines (DNHNES, 2018). Therefore, we used the data from the 2011–2016 survey cycles to ensure sufficient sample size. A total of 1423 nonpregnant participants (missing values of main variables less than 10%) were selected as our study population, and all of them tested the levels of urinary metals (**Appendix A Fig. S1**). In this study, all procedures were approved by the National Center for Health Statistics Research Ethics Review Board.

1.2. Measurements of Mo and Co

One-third of the adults were randomly selected for testing the levels of urinary metals. The urine specimens were stored at −70°C before testing. Urinary Mo and Co levels were measured using inductively coupled plasma mass spectrometers (ELAN® 6100 DRC^{Plus} or ELAN® DRC II, PerkinElmer Norwalk, USA). The limit of detection (LOD, μg/L) of urinary Mo and Co was 0.800 and 0.023, respectively. For those detections under LOD, were substituted by dividing the detection limit by the square root of 2 (CDC, 2011).

1.3. Measurements of FPG, HbA1c, insulin, and HOMA-IR

FPG was measured using the Roche Modular P chemistry analyzer (Roche Diagnostics, USA) in 2011–2012 survey cycle and Roche/Hitachi Cobas C Chemistry Analyzer in 2013–2016 survey cycles. HbA1c was measured using the Tosoh G8 Glycohemoglobin Analyzer (Tosoh Bioscience, Inc., CA). Insulin density was measured using the Elecsys 2010 analyzer (Roche Diagnostics Corporation, Indianapolis, USA) in 2011–2012 survey cycle and Tosoh AIA System analyzer (Tosoh Bioscience, Inc., CA) in 2013–2016 survey cycles. HOMA-IR was calculated on the basis of the FPG (mmol/L) and insulin (μU/mL) levels: HOMA = FPG × insulin ÷ 22.5 (Matthews et al., 1985).

1.4. Statistical analysis

The detailed characteristics of the study population were presented as number and percentage. Urinary Mo and Co levels were regarded as categorical variables (classified as quartiles, Q1 to Q4) or continuous variables (log-transformed to reduce skewness). Urine creatinine concentration was used to adjust the dilution of urine. The multiple linear regression model was used to explore the relationships between urinary Mo and Co and HbA1c, FPG, insulin, and HOMA-IR, which was further adjusted on the basis of age group, race/ethnicity, educational attainment, BMI, average daily energy intake, smoking status, alcohol consumption, history of hypertension, and family history of diabetes (single-metal model, **Appendix A Covariates**). The included covariates adjustment were based on the priori knowledge on the risk factors of diabetes, including socioeconomic status (Shrivastava et al., 2016), smoking status (Maddatu et al., 2017), excessive daily energy intake (Ojo, 2019), and alcohol consumption (Polsky and Akturk, 2017). The logistic regression model was used to explore the associations between urinary Mo and Co and diabetes risks. The dose-response relationships were explored by the restricted cubic spline model. The values of P_{trend} were obtained by including the median of each quartile (log10-transformed metal concentration) as a continuous variable (Yang et al., 2020). Moreover,

the sex-specific effects were investigated by hierarchical analysis.

Sensitivity analyses were further performed to add other metals in urine and illustrate the natural connection and mechanism of other metals in modifying the magnitude of associations (multiple-metal model). In addition, the BKMR model was used to analyze the interactions between urinary metals and diabetes-related biomarkers (Bellavia et al., 2019; Coker et al., 2018; Valeri et al., 2017). All of the statistical analyses in our study were performed by SPSS 20.0 (IBM, Armonk, NY, USA) and R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was defined as two-sided *P*-value <0.05.

2. Results

2.1. *Clinical and demographic characteristics of the study participants*

Table 1 shows the demographic characteristics of 1423 participants in this study: 804 (56.5%) males and 619 (43.5%) females. About 559 (39.3%) of the participants were 18–39 years, and more than half of the participants were college graduates (867, 60.9%) and never smoker (731, 51.4%). Of the participants, about 437 (30.7%) had a BMI < 25 kg/m^2, 295 (20.7%) had a family history of diabetes, and 567 (39.8%) had a history of hypertension. The median clinical values (25th, 75th) in the overall population were as follows: 5.55 (5.16, 6.05) mmol/L of FPG, 5.50% (5.20%, 5.80%) of HbA1c, 9.16 (5.70, 14.72) μU/mL of insulin, and 2.37 (1.39, 4.01) of HOMA-IR. **Appendix A Table S1** presents the comparisons of diabetes-related biomarker concentrations between this study and other studies.

Table 1. – Basic characteristics of adults from 2011-2016 U.S. NHANES in this study.

Variables	Males (*n*=804)	Females (*n*=619)	Overall (*n*=1423)
Age group, years			
18-39	313 (38.9%)	246 (39.7%)	559 (39.3%)
40-59	261 (32.5%)	205 (33.1%)	466 (32.7%)
≥ 60	230 (28.6%)	168 (27.1%)	398 (28.0%)
Race/ethnicity			
White	337 (41.9%)	278 (44.9%)	615 (43.2%)
Black	161 (20.0%)	120 (19.3%)	281 (19.7%)
Hispanic	191 (23.8%)	153 (24.7%)	344 (24.2%)
Other	115 (14.3%)	68 (11.0%)	183 (12.9%)
Educational attainment			
Less than high school	163 (20.3%)	83 (13.4%)	246 (17.3%)
High school diploma	193 (24.0%)	117 (18.9%)	310 (21.8%)
At least some college	448 (55.7%)	419 (67.7%)	867 (60.9%)
BMI, kg/m^{2}Educational attainmentw			
< 25.0	229 (28.5%)	208 (33.6%)	437 (30.7%)
25.0-29.9	312 (38.8%)	169 (27.3%)	481 (33.8%)
≥ 30	263 (32.7%)	242 (39.1%)	505 (35.5%)
Average daily energy intake, kJ			
Q1 (<1504)	159 (19.8%)	237 (38.3%)	396 (27.8%)
Q2 (1504-2068)	170 (21.1%)	172 (27.8%)	342 (24.0%)
Q3 (2068-2697)	211 (26.2%)	132 (21.3%)	343 (24.1%)
Q4 (>2697)	264 (32.8%)	78 (12.6%)	342 (24.0%)
Smoking status			
Never smoker	346 (43.0%)	385 (62.2%)	731 (51.4%)
Former smoker	245 (30.5%)	120 (19.4%)	365 (25.7%)
Current smoker	213 (26.5%)	114 (18.4%)	327 (23.0%)
Alcohol consumption, drinks/day			
≤ 1	236 (29.4%)	276 (44.6%)	512 (36.0%)
2	205 (25.5%)	193 (31.2%)	398 (28.0%)
≥ 3	363 (45.1%)	150 (24.2%)	513 (36.1%)
History of hypertension			
No	468 (58.2%)	388 (62.7%)	856 (60.2%)
Yes	336 (41.8%)	231 (37.3%)	567 (39.8%)
Family history of diabetes			
No	675 (84.0%)	453 (73.2%)	1128 (79.3%)
Yes	129 (16.0%)	166 (26.8%)	295 (20.7%)
FPG (mmol/L)	5.66 (5.27, 6.22)	5.38 (5.05, 5.86)	5.55 (5.16, 6.05)
HbA1c (%)	5.50 (5.30, 5.90)	5.50 (5.20, 5.80)	5.50 (5.20, 5.80)
Insulin (μU/mL)	9.21 (5.74, 14.98)	9.08 (5.62, 14.38)	9.16 (5.70, 14.72)
HOMA-IR	2.51 (1.43, 4.12)	2.03 (1.33, 3.80)	2.37 (1.39, 4.01)

NHANES: National Health and Nutrition Examination Survey; BMI: body mass index; Q: quartile; FPG: fasting plasma glucose; HbA1c: hemoglobin A1c; HOMA-IR: homeostasis model assessment for insulin resistance.
All continuous variables were presented as medians (interquartile ranges), and all categorical variables were presented as number (proportions).

2.2. *Overall and sex-specific associations between urinary Co and Mo levels and diabetes-related biomarkers*

Compared with the first quartile (Q1), urinary Co at the fourth quartile (Q4) was associated with elevated FPG in overall population ($\beta = 0.41$, 95% CI (confidence interval): 0.07–0.75) and males ($\beta = 0.61$, 95% CI: 0.17–1.04), with significantly increasing trends ($P = 0.007$ for overall population and $P = 0.033$ for males) (Table 2). Urinary Co at Q4 had high HbA1c levels in overall population (Q4 vs. Q1: $\beta = 0.22$, 95% CI: 0.04–0.40) and males (Q4 vs. Q1: $\beta = 0.31$, 95% CI: 0.09–0.54). Compared with that in Q1, urinary Co at Q4 was associated with elevated insulin levels ($\beta = 8.18$, 95% CI: 2.84–13.52) and elevated HOMA-IR levels ($\beta = 3.42$, 95% CI: 1.40–5.44) in males. When urinary Co was considered as a continuous variable, urinary Co was positively associated with insulin levels ($\beta = 5.20$, 95% CI: 0.89–9.50) and HOMA-IR levels ($\beta = 1.92$, 95% CI: 0.31–3.53) in overall population. For sensitivity analysis (**Appendix A Table S2**), compared with Q1, urinary Co at Q4 still had higher FPG levels ($\beta = 0.52$, 95% CI: 0.07–0.98), higher HbA1c levels ($\beta = 0.27$, 95% CI: 0.04–0.51), higher insulin levels ($\beta = 8.97$, 95% CI: 3.38–14.55), and higher HOMA-IR levels ($\beta = 3.57$, 95% CI: 1.46–5.68) in males. No significant relationship was found between urinary Co and the risk of diabetes (**Appendix A Table S2**).

Compared with Q1 (Table 3), urinary Mo at Q4 was associated with elevated FPG levels in the overall population ($\beta = 0.46$, 95% CI: 0.17–0.75), males ($\beta = 0.45$, 95% CI: 0.04–0.88), and females ($\beta = 0.42$, 95% CI: 0.02–0.82), with significantly increasing trends ($P = 0.001$ for overall population, $P = 0.029$ for males, and $P = 0.021$ for females). Compared with Q1, urinary Mo at Q4 was associated with elevated HbA1c levels in the overall population ($\beta = 0.27$, 95% CI: 0.11–0.42), males ($\beta = 0.22$, 95% CI: 0.01–0.44), and females ($\beta = 0.25$, 95% CI: 0.02–0.47), with significantly increasing trends ($P < 0.001$ for overall population, $P = 0.026$ for males, and $P = 0.012$ for females). When urinary Mo was regarded as a continuous variable, urinary Mo was positively associated with FPG levels and HbA1c in the overall population, males, and females (Table 3). Similar phenomenon was shown in sensitivity analysis (**Appendix A Table S3**). Significant relationships between urinary Mo and the risk of diabetes were found in the overall population (Q4 vs. Q1: OR=1.66, 95% CI: 1.07–2.58) and in males (Q4 vs. Q1: OR=1.82, 95% CI: 1.01–3.29) (**Appendix A Table S3**).

Table 2. – Adjusted β (95% CI) for diabetes-related biomarkers to quartiles of exposure for urinary Co from single-metal model.

Urinary Co ($\times 10^{-2}$ μg/g creatinine)	FPG	HbA1c	Insulin	HOMA-IR
Overall				
Q1 (<22.16)	Reference	Reference	Reference	Reference
Q2 (22.16-31.84)	0.47 (0.14, 0.79)	0.27 (0.10, 0.44)	0.81 (-2.55, 4.18)	0.59 (-0.67, 1.85)
Q3 (31.84-46.64)	0.32 (-0.01, 0.66)	0.10 (-0.08, 0.27)	1.54 (-1.94, 5.02)	0.67 (-0.64, 1.97)
Q4 (>46.64)	0.41 (0.07, 0.75)	0.22 (0.04, 0.40)	2.25 (-1.30, 5.79)	1.15 (-0.18, 2.48)
P trend	0.007	0.101	0.004	0.001
lg Co	0.38 (-0.03, 0.80)	0.11 (-0.11, 0.33)	5.20 (0.89, 9.50)	1.92 (0.31, 3.53)
Males				
Q1 (<20.11)	Reference	Reference	Reference	Reference
Q2 (20.11-26.61)	0.58 (0.16, 1.00)	0.26 (0.05, 0.48)	1.05 (-4.08, 6.18)	0.75 (-1.18, 2.69)
Q3 (26.61-37.45)	0.30 (-0.13, 0.73)	0.07 (-0.15, 0.29)	1.06 (-4.23, 6.34)	0.51 (-1.48, 2.51)
Q4 (>37.45)	0.61 (0.17, 1.04)	0.31 (0.09, 0.54)	8.18 (2.84, 13.52)	3.42 (1.40, 5.44)
P trend	0.033	0.039	0.004	0.002
lg Co	0.37 (-0.28, 1.01)	0.23 (-0.10, 0.55)	12.21 (4.42, 20.01)	4.44 (1.49, 7.39)
Females				
Q1 (<28.65)	Reference	Reference	Reference	Reference
Q2 (28.65-41.01)	-0.09 (-0.49, 0.31)	-0.08 (-0.31, 0.15)	-0.41 (-2.75, 1.92)	-0.16 (-0.98, 0.65)
Q3 (41.01-61.75)	-0.01 (-0.41, 0.39)	-0.05 (-0.28, 0.18)	-1.32 (-3.66, 1.02)	-0.55 (-1.36, 0.27)
Q4 (>61.75)	0.30 (-0.10, 0.70)	0.05 (-0.18, 0.27)	-1.35 (-3.70, 1.00)	-0.34 (-1.15, 0.48)
P trend	0.123	0.609	0.192	0.297
lg Co	0.31 (-0.21, 0.83)	-0.02 (-0.31,0.28)	-1.28 (-4.32, 1.75)	-0.43 (-1.48, 0.63)

Co: cobalt; lg Co: $\log_{10}$ Co; β: regression coefficient; 95% CI: 95% confidence intervals.

Table 3. – Adjusted β (95% CI) for diabetes-related biomarkers to quartiles of exposure for urinary Mo from single-metal model.

Urinary Mo (μg/g creatinine)	FPG	HbA1c	Insulin	HOMA-IR
Overall				
Q1 (<23.92)	Reference	Reference	Reference	Reference
Q2 (23.92-35.02)	0.05 (-0.23, 0.34)	0.07 (-0.08, 0.22)	-1.31 (-4.28, 1.67)	-0.26 (-1.38, 0.85)
Q3 (35.02-48.71)	0.20 (-0.09, 0.49)	0.18 (0.03, 0.34)	-1.42 (-4.42, 1.57)	-0.32 (-1.45, 0.80)
Q4 (>48.71)	0.46 (0.17, 0.75)	0.27 (0.11, 0.42)	-1.58 (-4.62, 1.46)	0.15 (-0.99, 1.29)
P trend	0.001	<0.001	0.321	0.841
lg Mo	0.60 (0.19, 1.02)	0.37 (0.15, 0.59)	-1.83 (-6.13, 2.48)	0.14 (-1.47, 1.76)
Males				
Q1 (<22.26)	Reference	Reference	Reference	Reference
Q2 (22.26-32.77)	0.04 (-0.37, 0.45)	0.04 (-0.17, 0.25)	-2.49 (-7.50, 2.52)	-0.49 (-2.39, 1.40)
Q3 (32.77-46.26)	0.16 (-0.25, 0.57)	0.13 (-0.08, 0.34)	-2.61 (-7.62, 2.40)	-0.75 (-2.64, 1.15)
Q4 (>46.26)	0.45 (0.04, 0.86)	0.22 (0.01, 0.44)	-1.86 (-6.94, 3.21)	0.29 (-1.63, 2.21)
P trend	0.029	0.026	0.482	0.849
lg Mo	0.67 (0.09, 1.24)	0.35 (0.06, 0.65)	-2.38 (-9.38, 4.62)	0.27 (-2.38, 2.92)
Females				
Q1 (<25.79)	Reference	Reference	Reference	Reference
Q2 (25.79-37.39)	-0.06 (-0.45, 0.33)	0.01 (-0.21, 0.23)	0.35 (-1.94, 2.64)	-0.07 (-0.86, 0.73)
Q3 (37.39-51.80)	0.18 (-0.22, 0.57)	0.19 (-0.03, 0.41)	0.97 (-1.33, 3.26)	0.38 (-0.42, 1.18)
Q4 (>51.80)	0.42 (0.02, 0.82)	0.25 (0.02, 0.47)	-1.38 (-3.73, 0.96)	-0.12 (-0.94, 0.70)
P trend	0.021	0.012	0.364	0.937
lg Mo	0.49 (-0.10, 1.08)	0.36 (0.02, 0.69)	-0.65 (-4.10, 2.81)	0.12 (-1.08, 1.32)

Mo: molybdenum; lg Mo: $\log_{10}$ Mo.

2.3. *Dose-response associations between urinary Co and Mo levels and diabetes-related biomarkers*

As shown in Fig. 1, positive linear dose-response relationships between urinary Co and insulin levels ($P_{overall}$ <0.001, $P_{nonlinear}$ = 0.513) and HOMA-IR ($P_{overall}$ = 0.001, $P_{nonlinear}$ = 0.736) were observed in males. In the overall population, urinary Mo was positively correlated with FPG levels ($P_{overall}$ = 0.028, $P_{nonlinear}$ = 0.826) and HbA1c levels ($P_{overall}$ = 0.034, $P_{nonlinear}$= 0.376). Furthermore, no significant

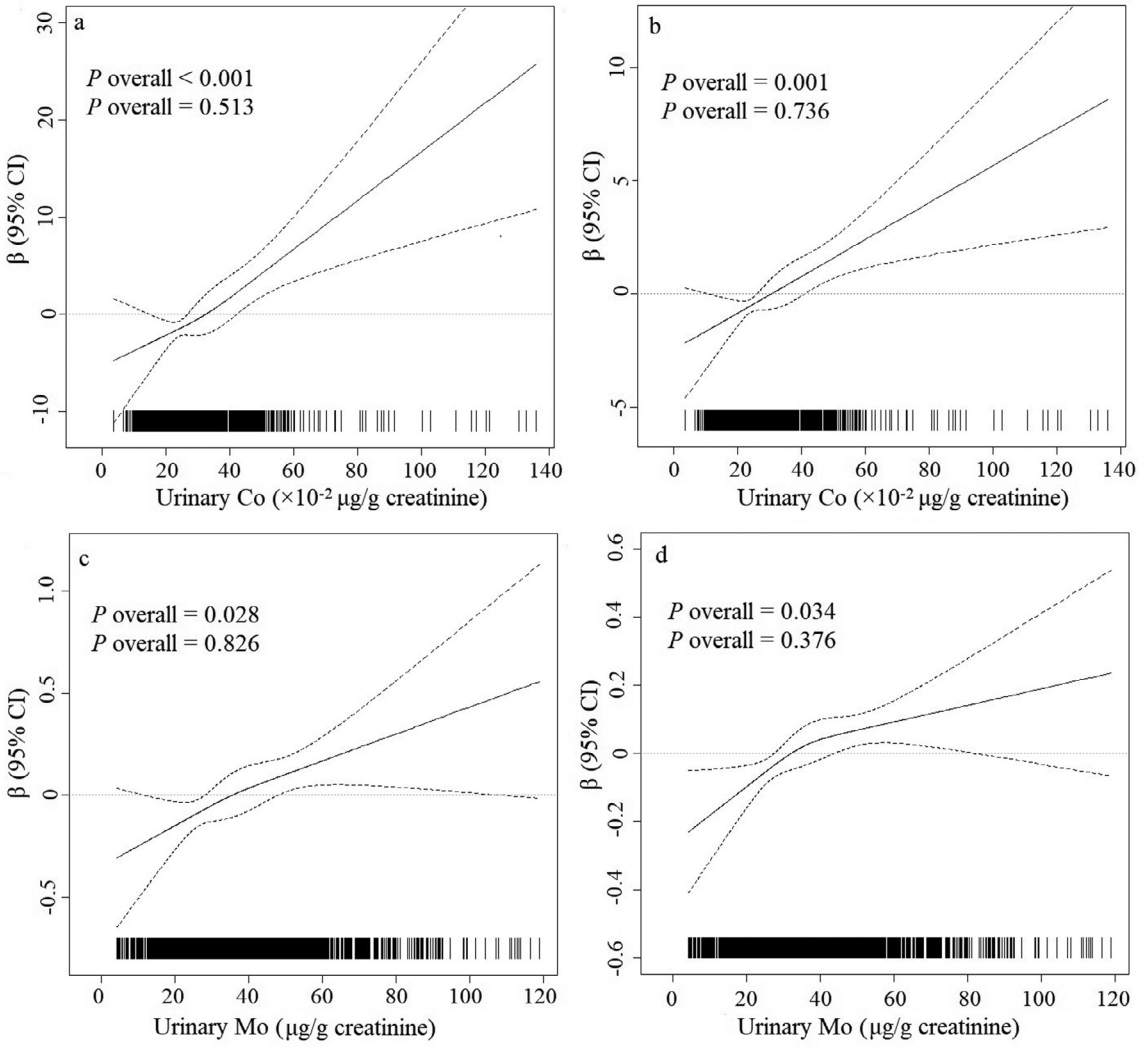

Fig. 1. – The does-response relationship between urinary Co, Mo and diabetes-related biomarkers by spline analysis. (a) Urinary Co and insulin in males; (b) Urinary Co and HOMA-IR in males; (c) Urinary Mo and FPG in the overall population; (d) Urinary Mo and HbA1c in the overall population.

dose-response relationships between urinary Co and Mo and other diabetes-related biomarkers were found (**Appendix A Table S4, Figs. S2-S3**).

2.4. *Interaction of multiple urinary metals on diabetes-related biomarkers*

When the concentrations of Mo and Cd were fixed at the median, the slope of the dose-response curve of urinary Mn and FPG increased with the increase of urinary Co from 10th to 90th (**Appendix A Fig. S4a)**, which indicated the interaction between Mn and Co on FPG. However, when the concentrations of the other two metals were fixed at the median, the dose-response curve of the abscissa metal with HbA1c, insulin, or HOMA-IR levels remained parallel or overlapped with the increase of the metal concentration on the right coordinate from 10th to 90th, indicating no interaction between urinary metals and HbA1c, insulin, and HOMA-IR (**Appendix A Fig. S4b–d**).

3. Discussion

Our study found that high Co levels were strongly correlated with increased levels of FPG, HbA1c, insulin, and HOMA-IR in male participants. Meanwhile, high Mo levels may elevate FPG and HbA1c levels without evident gender differentials. The interactions of multiple metals indicated that Mn and Co may synergistically increase the FPG level in our study population.

In this study, the concentration of urinary Co was significantly lower in males when compared with female. Similar results of the gender-differential of urinary Co were also observed by Yang et al. (2017) that the urinary Co concentration (0.31 μg/L) in males was significantly lower than that in females (0.84 μg/L). Meanwhile, our results showed that high urinary Co levels were highly correlated with increased FPG, HbA1c, insulin, and HOMA-IR in males. In addition, Agarwal et al. (2011) reported that high urinary Co level was associated with increased risk of diabetes in males. These

gender differentials in Co concentration and Co-related risks of diabetes may link to the sex hormone. Diabetes risk was found to be connected to gender in former studies, and endogenous hormones had a substantial effect on the sex specificity between urinary Co concentrations and diabetes-related biomarkers (Ge et al., 2021). Moreover, Ding et al. (2006) found that high testosterone level was positively associated with diabetes risk in females, but high testosterone level had an inverse association with diabetes risk in males. Similarly, studies (Aldhoon-Hainerova et al., 2017; Liu and Sun, 2018) have found gender variations in androgen effects on insulin resistance: decreased androgen levels can increase insulin resistance in males, but decreased androgen levels had adverse effects in females. High Co could lead to insulin resistance. According to Lai et al. (2018), Co served as a bio-functional component of vitamin B_{12}, which could lead to insulin resistance and diabetes at low levels. Furthermore, as shown by existing experimental evidence, Co had a potential hypoglycemic effect, which may prevent the development of diabetes (Nomura et al., 2005). Co could promote the formation of free radicals, resulting in lipid peroxidation (Mao et al., 1996). Moreover, Co toxicity may lead to mitochondrial dysfunction (Karovic et al., 2007), which played a key role in the development of diabetes (Rovira-Llopis et al., 2017). However, most of these mechanism studies were based on animal experiments; thus, any future studies on the Co-related mechanism of diabetes were still strongly warranted.

Mo is an essential trace element that plays a vital role in the human health. As a cofactor of several enzymes in the human body, including xanthine dehydrogenase (XDH) and xanthine oxidase, any abnormality of the endogenous or exogenous balance of Mo could disrupt the normal function of the Mo-related enzyme systems (Novotny and Peterson, 2018). However, increased XDH activity may lead to uric acid accumulation, gout attacks, and reactive-oxygen-species-related diseases (Ichida et al., 2012). Based on existing evidence, increased XDH activity and hyperuricemia have been observed in cardiovascular diseases, anemia, and complications of diabetes(ATSDR, 2020)-. In addition, the role of Mo was manifested in protein synthesis, metabolism, and growth (Mehri and Marjan, 2013). By promoting protein catabolism, Mo can metabolize fat and carbohydrates, thereby providing energy and vitality to the body **(ATSDR, 2020)**. In this study, high urinary Mo exposure could be a risk factor for increased FPG and HbA1c levels in males and females. Xiao et al. (2018) and Feng et al. (2015) reported that high urinary Mo concentration could increase the risk of diabetes. Sun et al. (2012) showed that Mo was related to HOMA-IR. Rotter et al. (2015) found that Mo concentration was positively correlated with insulin levels. However, the relationships between urinary Mo concentration and insulin and HOMA-IR were not significant in our study. This result might be due to the age difference when compared with the above-mentioned studies. In our study, we included U.S. adults aged 18 years and above. By contrast, Rotter et al. (2015) only included male aged 50–75 years, and Sun et al. (2012) recruited participants aged 35–54 years in the middle- and high-income areas. Several studies, particularly the prospective ones, were worthy of further investigation.

Although previous studies (Feng et al., 2015; Yuan et al., 2018) have investigated the relationships between multiple-metal exposure and diabetes-related biomarkers, metal mixtures may be linked to diabetes-related biomarkers with complicated non-linearity and possible multicollinearity. These difficulties cannot be solved by traditional multivariate parameter regression models. Therefore, based on the NHANES database, the present study used the BKMR model to explore the interaction between urinary metals and diabetes-related biomarkers. Our results showed that the interactions between Mn and Co may had a strong impact on the FPG level. To date, no studies have explored the interaction between urinary Co, Mo, and other metals with diabetes-related biomarkers. However, previous studies found interactions between arsenic and selenium (Li et al., 2019) as well as Co and nickel (Yang et al., 2017) and FPG and between Mo and copper (Flores et al., 2011) and diabetes-related complications. Heavy metals competed with base metal ions in enzyme molecules, producing structural and functional alterations in these biomolecules and interfering with body's metal balance (Koedrith and Seo, 2011). Zheng et al. (2018) hypothesized that oxidative stress induced by toxic metals would lead to the destruction or dysfunction of pancreatic β-cell, whereas trace essential metals possessed antioxidant properties at normal levels, which could offset oxidative stress induced by toxic metals, such as Zn/Cu and Se/Cd. These studies showed that the interaction of metal exposure could play a nonnegligible role in the development of diabetes.

Our study has some strengths. First, the U.S. NHANES is large-scale research with strict protocols and extensive quality control procedures, which is a major strength in this study. Second, urinary creatinine concentration was adjusted to control urine dilution. In addition, this study used the BKMR model to study the interaction between urinary metals and diabetes-related biomarkers, avoiding the limitations of traditional statistical methods caused by the potential multicollinearity or complex nonlinear correlation between two or more metals. However, some limitations in our study should also be referred. First, confounding factors caused by unknown or unmeasured variables were impossible to completely tease out. However, based on previous reports, most of the known risk factors of diabetes in the NHANES database were fully adjusted in our regression models, which may minimize residual confounding. Second, NHANES is cross-sectional research that struggled to establish causal inference, which is similar to other cross-sectional studies. Third, urinary metals were measured in one-time spot urine, which may only indicate the temporal changes of these chemicals in urine. Furthermore, heavy metals, such as lead, cadmium, and mercury, may increase the risk of diabetes. Therefore, the relationship between heavy metals and diabetes should be considered in future studies.

4. Conclusion

The present study showed the sex-specific effects of urinary Co on diabetes-related biomarkers: high urinary Co levels were associated with increased FPG, HbA1c, insulin, and HOMA-IR levels in males but not in females, and positive lin-

ear dose-response relationships were observed between urinary Co and insulin and HOMA-IR. Urinary Mo had no gender differentials on diabetes-related biomarkers: high urinary Mo levels were associated with elevated FPG and HbA1c levels in males and females, and positive linear dose-response relationships were observed between the two population groups. The interactions between Mn and Co may have a strong impact on the FPG level.

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Appendix A Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jes.2021.10.023.

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