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ORIGINAL ARTICLE

Effects of multi-metal exposure on the risk of diabetes mellitus among people aged 40–75 years in rural areas in southwest China

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ABSTRACT

Aims/Introduction: Metals play an important role in diabetes mellitus. This cross-sectional study aimed to evaluate the overall, individual and interactive effects of multi-metal exposure on the prevalence of diabetes mellitus, impaired fasting glucose (IFG) rate and fasting blood glucose (FBG) levels.

Materials and Methods: The FBG levels of a study population from a cadmium (Cd)-polluted area (n = 250) and an unpolluted area (n = 204), and the metal levels, including magnesium, calcium (Ca), iron (Fe), zinc (Zn), arsenic (As), Cd, copper and lead (Pb) in blood and urine were detected. The study population was divided into a normal fasting glucose group, an IFG group and a diabetes mellitus group on the basis of FBG levels.

Results: The IFG rate and diabetes mellitus prevalence were negatively associated with blood Cd and urine Zn levels (IFG rate: odds ratio [OR] 0.780, 95% confidence interval [CI] 0.655–0.928; OR 0.622, 95% CI 0.465–0.831. Diabetes mellitus prevalence: OR 0.506, 95% CI 0.288–0.888; OR 0.609, 95% CI 0.395–0.939), the IFG rate was positively associated with urine Fe levels (OR 1.876, 95% CI 1.290–2.778), and diabetes mellitus prevalence was positively associated with urine Pb and blood Fe levels (OR 1.185, 95% CI 1.022–1.376; OR 1.008, 95% CI 1.001–1.014). A linear negative correlation was observed between FBG levels and blood Cd, and non-linear inverted U-shaped associations were found between FBG levels and Zn, Pb and copper in urine.

Conclusions: This research suggests that multi-metal exposure, especially Cd, Fe, Zn, copper and Pb, is linked to diabetes mellitus, and the interactive effects of multiple metals require further exploration.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia with disorders in insulin secretion and/or utilization caused by multiple etiologies. It is one of the major causes of renal failure, stroke, disability and other cardiovascular diseases[1], and results in a considerable disease burden and economic loss. In the past 40 years, diabetes mellitus prevalence in China has increased by nearly 17-fold from 0.67% in 1980 to 11.2% in 2020[2]. Diabetes mellitus has become a major public health problem in China. Fasting blood glucose (FBG) is not only a basic diagnostic index, but also a known independent factor that affects diabetes mellitus development[3]. Research has shown that individuals with impaired fasting glucose (IFG) have a 20–30% chance of developing diabetes mellitus over the next 5–10 years[4]. Compared with other tests (e.g., glycosylated hemoglobin), the FBG test is less expensive and easier to perform; these features are vital for the primary prevention of diabetes, especially in low- and middle-income countries[5]. Therefore, exploring IFG- and diabetes mellitus-related risk factors is important for establishing targeted prevention and treatment strategies.

Traditional risk factors, such as heredity and unhealthy diet patterns, cannot fully explain the etiology and high prevalence

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of diabetes mellitus. Thus, the interest in non-traditional and novel risk factors, such as metal exposure, is increasing. Heavy metals can affect mitochondrial mechanisms and increase the production of free radicals, leading to oxidative stress and inflammation, both of which might play a role in metabolic disorders, such as diabetes mellitus or obesity[6]. However, the effects of metal exposure, especially mixed exposure to multiple metals, on diabetes mellitus development have not been fully studied[7]. Most previous studies on the association between metal exposure and elevated FBG or diabetes mellitus risk have focused on single-metal exposure models[8], which might ignore the problem of strong correlation or even collinearity between metals and the impact of metal interactions on health[7]. Although several recent studies have explored the relationship between multi-metal exposure and diabetes mellitus risk, their conclusions are inconsistent. Several population-based epidemiological surveys from different regions have found that magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), arsenic (As), cadmium (Cd), copper (Cu) and lead (Pb) are associated with FBG or diabetes mellitus prevalence[7,9–15], whereas other studies have found no association[3,16–19]. Furthermore, the interaction of metals presents a challenge for correlation exploration. Thus, further research is required to explore the role of multi-metal exposure in the onset and development of diabetes mellitus.

Given the common correlation and even collinearity among metals in mixed exposure, complex non-linear and non-additive relationships or interactive effects might be exerted on health outcomes due to the mixed exposure, and traditional multivariate regression methods have certain limitations in discovering these associations[20]. Therefore, a new statistical method called Bayesian kernel machine regression (BKMR) was applied in the present study; BKMR has been used to estimate the health effects of mixtures of various pollutants[21–23]. BKMR can effectively reflect the individual effects of each metal on health outcomes under multi-metal exposure and evaluate the interactive effects of different metals.

Furthermore, we assessed the internal exposure levels to eight metal elements (i.e., Mg, Ca, Fe, Zn, As, Cd, Cu and Pb) of people aged 40–75 years in Cd-polluted and unpolluted rural areas in southwest China. We examined the correlation between the various metals and diabetes mellitus prevalence, IFG rate, and FBG levels in the case of multi-metal exposure. The present study is expected to fill the gap in the research on the relationship between multi-metal exposure and diabetes mellitus in southwest China, and provide new insights into the prevention and treatment of diabetes mellitus.

MATERIALS AND METHODS

Study sites and population

The selected study areas were grouped into a Cd-polluted area where the Cd levels in rice were >0.2 mg/kg and an unpolluted area where the Cd levels in rice were <0.05 mg/kg[24]. The two groups have similar economic conditions, geographical environments, lifestyles and eating habits. The inclusion and exclusion criteria were as follows:

Inclusion criteria: (i) residential duration ≥15 years; (ii) age 40–75 years; and (iii) living on rice, vegetables and water in the survey areas.

Exclusion criteria: (i) abnormal metal exposure levels (three-fold higher than the 99th percentile of each metal); (ii) family genetic history of diabetes mellitus; (iii) suffering from hyperthyroidism liver, chronic gastrointestinal and kidney diseases; (iv) taking drugs that affect glucose metabolism, including oral antidiabetic agents or insulin; and (v) the data on FBG or metal exposure in blood and urine samples are missing.

Data collection

The basic information, including demographic information, health status and living habits, of the research participants was collected through questionnaires. Body mass index was calculated as the ratio of bodyweight divided by the square of height (kg/m^2). Smoking status was defined as current smokers who currently smoked at least one cigarette a day and had been smoking for more than a month, former smokers who had quit smoking for >6 months, and non-smokers who had never smoked.

Blood and urine sample collection

First-morning mid-urinary and fasting blood samples were collected in plastic bottles and anticoagulant vacuum blood collection tubes, respectively, and aliquoted into 1.5-mL cryotubes, then transported to the laboratory under refrigeration. The samples were stored at −80°C until analysis.

Indicator measurement in blood and urine samples

Inductively coupled argon plasma mass spectrometry was carried out with an Agilent 7700 instrument (Agilent Technologies, Santa Clara, CA, USA) to determine the concentrations of Mg, Ca, Fe, Zn, As, Cd, Cu and Pb in the blood and urine samples (the concentrations are referred to as BMg, BCa, BFe, BZn, BAs, BCd, BCu and BPb, as well as UMg, UCa, UFe, UZn, UAs, UCd, UCu and UPb, respectively, in the following text). The limit of quantification for blood and urine metals was ≤0.01 μg/L. Seronorm™ Trace Elements Urine 0511545, Seronorm™ Trace Elements Urine Blank and Seronorm™ Trace Elements Whole Blood Level 1–2 (Nycomed Pharma AS, Oslo, Norway) were used as standard reference materials. Quality control procedures were also carried out. For example, each batch of samples was detected with three blank samples and a repeatedly injected standard material sample at the same time.

The FBG levels and urine creatinine were measured with a Hitachi 7180 automatic biochemical analyzer and the picric acid method using a commercial assay kit (Roche, Basel, Switzerland), respectively. The metal levels in urine were corrected by urinary creatinine levels to minimize the influence of urine amount and expressed as mg/g Cr or μg/g Cr.

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fication of FBG status",5,2,1,0,100mm,100mm,100mm,100mm>Classification of FBG status

The FBG status of the study population was divided into a normal fasting glucose (NFG) group, an IFG group and a diabetes mellitus group. NFG, IFG and diabetes mellitus were defined based on the criteria of the American Diabetes Association; that is, FPG <5.6 mmol/L, 5.6 ≤ FPG < 7.0 mmol/L and FBG ≥7.0 mmol/L or a self-reported physician diagnosis (with or without FBG ≥7.0 mmol/L), respectively. IFG rate was calculated as the percentage of the number of IFG groups divided by the total population of the study (%), and diabetes mellitus prevalence was calculated as the percentage of the number of diabetes mellitus groups divided by the total population of the study (%).

Statistical analysis

The data were entered using the double-entry method in Epidata software after rechecking, and statistical packages (including SPSS 21.0 [IBM Corp., Armonk, NY, USA] and R 4.1.0 [The R Foundation for Statistical Computing, Vienna, Austria] software) were used for data analysis.

Normal distribution, non-normal distribution and categorical data are presented as the mean ± standard deviation, median (25th and 75th percentile) and quantity (percent), respectively. The differences between the two research sites in demographic characteristics, FBG levels, metal exposure levels, IFG rate and diabetes mellitus prevalence were compared through a *t*-test, Mann–Whitney *U*-test or χ^2-test, and the correlations among the eight metals were analyzed using Spearman's rank correlation. The associations among metal levels in blood and urine, IFG rate, and diabetes mellitus prevalence were analyzed using unconditional logistic regression. BKMR was used to analyze the overall, individual and interactive effects of the metals on FBG levels. $P < 0.05$ was considered statistically significant.

RESULTS

Participant characteristics

The demographic characteristics and metal exposure levels of the study population are shown in Tables 1 and 2. A total of 454 participants, including 204 participants from an unpolluted area and 250 participants from a Cd-polluted area, were recruited. A significant difference in FBG status was observed between the two areas and the FBG levels in the unpolluted area were higher ($P < 0.05$). Compared with the participants in the unpolluted area, those in the Cd-polluted area had higher levels of BMg, BAs, BCd and UCd ($P < 0.05$), but their levels of BCa, BFe, BZn, BCu, UMg, UCa, UFe, UZn and UAs were lower ($P < 0.05$), which implies that a high Cd-body burden might adversely affect other beneficial minerals. No significant differences between the two areas were found in terms of age, sex, body mass index and smoking status ($P > 0.05$).

Table 1 | Characteristics of the study population

Variables	Unpolluted area (n = 204)	Cd-polluted area (n = 250)	*P*-value
Age (years)	58.50 ± 8.90	57.98 ± 8.68	0.521
BMI (kg/m^2)	24.56 ± 3.47	24.41 ± 3.22	0.639
FBG (mmol/L)	5.85 ± 1.62	4.95 ± 1.61	0.000*
Sex, *n* (%)			
Male	88 (43.14)	100 (40.00)	0.500
Female	116 (56.86)	150 (60.00)	
Smoking status, *n* (%)			
Non-smoker	126 (61.77)	154 (61.60)	0.995
Current smoker	59 (28.92)	72 (28.80)	
Former smoker	19 (9.31)	24 (9.60)	
FBG status, *n* (%)			
NFG group	115 (56.4)	171 (68.4)	0.022*
IFG group	69 (33.8)	57 (22.8)	
DM group	20 (9.80)	22 (8.80)	

Total n = 454. *$P < 0.05$. BMI, body mass index; DM, diabetes mellitus; FBG, fasting blood glucose; IFG, impaired fasting glucose; NFG, normal fasting glucose.

's rank correlation",5,2,1,0,100mm,100mm,100mm,100mm>Spearman's rank correlation

The correlations between the different metals in the blood and urine samples are shown in Tables 3–6. Significant associations between most metal levels were found in blood and urine in both areas ($P < 0.05$ or $P < 0.01$). For example, UMg and UCa had a positive correlation, whereas BCa and BAs had a negative correlation in both areas. Particularly, BZn and BFe, as well as BZn and BCu, had a strong positive correlation in the unpolluted area ($r_s = 0.815$, $P < 0.01$; $r_s = 0.909$, $P < 0.01$).

Logistic regression analysis

Table 7 presents the logistic regression models for IFG associated with the multiple metals. After adjusting for potential confounding factors, the regression analysis showed a negative association between BCd levels and IFG rate in the unpolluted area (odds ratio [OR] 0.780, 95% confidence interval [CI] 0.655–0.928). A positive association between UFe levels and IFG rate, and a negative association between UZn levels and IFG rate were found in the Cd-polluted area (OR 1.876, 95% CI 1.290–2.778 for UFe; OR 0.622, 95% CI 0.465–0.831 for UZn).

Table 8 presents the logistic regression models for diabetes mellitus associated with multiple metals. After adjusting for potential confounding factors, the regression analysis showed a negative association between BCd levels and diabetes mellitus prevalence in both regions (OR 0.506, 95% CI 0.288–0.888 for the unpolluted area; OR 0.722, 95% CI 0.536–0.972 for the Cd-polluted area). We also found positive associations of diabetes mellitus prevalence with BFe and UPb levels in the unpolluted area (OR 1.008, 95% CI 1.001–1.014 for BFe; OR 1.185, 95% CI 1.022–1.376 for UPb), and a negative association between

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Table 2 | Blood and urinary levels of eight metals/metalloids

Exposure measure	Unpolluted area (n = 204) Median (25th, 75th percentile)	Cd-polluted area (n = 250) Median (25th, 75th percentile)	P-value
BMg (mg/L)	25.60 (16.92, 40.27)	35.60 (32.08, 40.50)	0.000*
BCa (mg/L)	76.90 (48.23, 92.88)	65.85 (58.98, 74.08)	0.028*
BFe (mg/L)	599.00 (440.25, 755.00)	465.00 (413.50, 538.75)	0.000*
BZn (mg/L)	5.77 (3.79, 9.51)	4.98 (4.18, 5.86)	0.001*
BAs (μg/L)	3.97 (0.50, 8.98)	6.44 (3.18, 8.73)	0.000*
BCd (μg/L)	1.90 (1.09, 4.69)	4.09 (2.88, 6.97)	0.000*
BCu (μg/L)	933.50 (666.75, 1420.00)	755.50 (695.75, 900.75)	0.000*
BPb (μg/L)	28.30 (17.50, 41.03)	32.30 (19.03, 44.20)	0.110
UMg (mg/g Cr)	104.49 (72.96, 175.26)	86.99 (55.24, 123.00)	0.000*
UCa (mg/g Cr)	243.55 (164.40, 366.65)	163.80 (90.78, 263.23)	0.000*
UFe (mg/g Cr)	2.76 (1.61 ,4.63)	1.05 (0.53, 2.05)	0.000*
UZn (mg/g Cr)	0.57 (0.38, 0.89)	0.42 (0.29, 0.64)	0.000*
UAs (μg/g Cr)	25.15 (15.92, 42.09)	15.21 (10.03, 21.77)	0.000*
UCd (μg/g Cr)	2.17 (1.29, 3.21)	4.96 (3.43, 7.12)	0.000*
UCu (μg/g Cr)	12.24 (6.45, 36.91)	18.02 (9.27, 27.89)	0.870
UPb (μg/g Cr)	1.64 (0.74, 3.96)	1.54 (0.66, 3.73)	0.544

*P < 0.05. BAs, concentration of arsenic in the blood; BCa, concentration of calcium in the blood; BCd, concentration of cadmium in the blood; BCu, concentration of copper in the blood; BFe, concentration of iron in the blood; BMg, concentration of magnesium in the blood; BPb, concentration of lead in the blood; BZn, concentration of zinc in the blood; UAs, concentration of arsenic in the urine; UCa, concentration of calcium in the urine; UCd, concentration of cadmium in the urine; UCu, concentration of copper in the urine; UFe, concentration of iron in the urine; UMg, concentration of magnesium in the urine; UPb, concentration of lead in the urine; UZn, concentration of zinc in the urine.

Table 3 | Spearman's rank correlation coefficients between blood metals in the unpolluted area

	BMg (mg/L)	BCa (mg/L)	BFe (mg/L)	BZn (mg/L)	BAs (μg/L)	BCd (μg/L)	BCu (μg/L)	BPb (μg/L)
BMg (mg/L)	1.000	0.511**	0.035	–0.231**	–0.439**	–0.274**	–0.273**	0.504**
BCa (mg/L)		1.000	0.139*	–0.135	–0.250**	–0.003	–0.014	0.328**
BFe (mg/L)			1.000	0.815**	0.315**	0.431**	0.739**	0.362**
BZn (mg/L)				1.000	0.359**	0.489**	0.909**	0.177*
BAs (μg/L)					1.000	0.319**	0.337**	0.013
BCd (μg/L)						1.000	0.425**	0.190**
BCu (μg/L)							1.000	0.091
BPb (μg/L)								1.000

**P < 0.01; *P < 0.05. BAs, concentration of arsenic in the blood; BCa, concentration of calcium in the blood; BCd, concentration of cadmium in the blood; BCu, concentration of copper in the blood; BFe, concentration of iron in the blood; BMg, concentration of magnesium in the blood; BPb, concentration of lead in the blood; BZn, concentration of zinc in the blood.

diabetes mellitus prevalence and UZn levels in the Cd-polluted area (OR 0.609, 95% CI 0.395–0.939).

BKMR analyses

The effects of mixed metals, a single metal and metal-to-metal interaction on FBG levels under the multi-metal exposure scenario are shown in Figures 1–4. For the unpolluted area, we did not find any association between blood metals and FBG (Figure 1a–d). When urine metals were used as the exposure variables, the FBG levels increased when the levels of all metals were higher than their 55th percentile (Figure 2a). A significant non-linear correlation was found between UZn and UPb, as well as UCu and FBG, which positively correlated with FBG when the metal levels were low, and negatively correlated with FBG when the concentrations were high (Figure 2b and c). The UPb exposure–response curve gradually became steeper with the increase in UZn concentration when UPb was at a

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Table 4 | Spearman's rank correlation coefficients between urine metals in the unpolluted area

	UMg (mg/gCr)	UCa (mg/g Cr)	UFe (mg/g Cr)	UZn (mg/g Cr)	UAs (mg/g Cr)	UCd (mg/g Cr)	UCu (mg/g Cr)	UPb (mg/g Cr)
UMg (mg/g Cr)	1.000	0.795**	0.458**	0.602**	0.558**	0.259**	0.068	0.327**
UCa (mg/g Cr)		1.000	0.590**	0.573**	0.479**	0.285**	0.246**	0.405**
UFe (mg/g Cr)			1.000	0.438**	0.287**	0.199**	0.453**	0.371
UZn (mg/g Cr)				1.000	0.405**	0.248**	0.256**	0.386**
UAs (μg/g Cr)					1.000	0.281**	0.124	0.390**
UCd (μg/g Cr)						1.000	0.295**	0.261**
UCu (μg/g Cr)							1.000	0.430**
UPb (μg/g Cr)								1.000

**$P < 0.01$; *$P < 0.05$. UAs, concentration of arsenic in the urine; UCa, concentration of calcium in the urine; UCd, concentration of cadmium in the urine; UCu, concentration of copper in the urine; UFe, concentration of iron in the urine; UMg, concentration of magnesium in the urine; UPb, concentration of lead in the urine; UZn, concentration of zinc in the urine.

Table 5 | Spearman's rank correlation coefficients between blood metals in the cadmium-polluted area

	BMg (mg/L)	BCa (mg/L)	BFe (mg/L)	BZn (mg/L)	BAs (μg/L)	BCd (μg/L)	BCu (μg/L)	BPb (μg/L)
BMg (mg/L)	1.000	0.163**	0.348**	0.449**	0.129*	0.108	0.292**	0.075
BCa (mg/L)		1.000	0.126*	−0.064	−0.226**	0.190**	0.329**	0.255**
BFe (mg/L)			1.000	0.643**	0.050	0.262**	0.201**	0.508**
BZn (mg/L)				1.000	0.113	0.287**	0.439**	0.227**
BAs (μg/L)					1.000	0.004	0.064	−0.208**
BCd (μg/L)						1.000	0.242**	0.270**
BCu (μg/L)							1.000	0.040
BPb (μg/L)								1.000

**$P < 0.01$; *$P < 0.05$. BAs, concentration of arsenic in the blood; BCa, concentration of calcium in the blood; BCd, concentration of cadmium in the blood; BCu, concentration of copper in the blood; BFe, concentration of iron in the blood; BMg, concentration of magnesium in the blood; BPb, concentration of lead in the blood; BZn, concentration of zinc in the blood.

Table 6 | Spearman's rank correlation coefficients between urine metals in the cadmium-polluted area

	UMg (mg/gCr)	Uca (mg/g Cr)	UFe (mg/g Cr)	UZn (mg/g Cr)	UAs (mg/g Cr)	UCd (mg/g Cr)	UCu (mg/g Cr)	UPb (mg/g Cr)
UMg (mg/g Cr)	1.000	0.643**	0.508**	0.341**	0.397**	**	0.323**	0.308**
Uca (mg/g Cr)		1.000	0.682**	0.237**	0.275**	0.198**	0.287**	0.352**
UFe (mg/g Cr)			1.000	0.214**	0.242**	0.011	0.312**	0.217**
UZn (mg/g Cr)				1.000	0.172**	0.174**	0.333**	0.259**
UAs (μg/g Cr)					1.000	0.297**	0.424**	0.257**
UCd (μg/g Cr)						1.000	0.301**	0.187**
UCu (μg/g Cr)							1.000	0.349**
UPb (μg/g Cr)								1.000

**$P < 0.01$; *$P < 0.05$. UAs, concentration of arsenic in the urine; UCa, concentration of calcium in the urine; UCd, concentration of cadmium in the urine; UCu, concentration of copper in the urine; UFe, concentration of iron in the urine; UMg, concentration of magnesium in the urine; UPb, concentration of lead in the urine; UZn, concentration of zinc in the urine.

high concentration, indicating that a positive interactive effect possibly occurred between UZn and UPb (Figure 2d).

For the Cd-polluted area, we found a negative correlation between BCd and FBG, but no overall effect of blood mixed metal on FBG was found (Figure 3a–c). In the bivariate exposure–response function, the exposure–response curve of BCa gradually flattened with the increase in the BCd level. Hence, BCd and BCa might exert an interactive effect on FBG level (Figure 3d). When urine metals were used as the exposure variables, we found a positive correlation between the metal

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Table 7 | Logistic regression models for impaired fasting glucose associated with multiple metals

	Unpolluted area (n = 184)		Cd-polluted area (n = 228)	
	OR (95% CI)	P-value	OR (95% CI)	P-value
BMg (mg/L)	0.960 (0.916, 1.007)	0.092	1.046 (0.985, 1.111)	0.145
BCa (mg/L)	0.997 (0.980, 1.015)	0.770	1.002 (0.971, 1.034)	0.902
BFe (mg/L)	1.003 (0.999, 1.006)	0.112	1.003 (0.998, 1.008)	0.255
BZn (mg/L)	0.876 (0.752, 1.020)	0.089	0.939 (0.597, 1.479)	0.787
BAs (μg/L)	1.011 (0.994, 1.028)	0.195	1.031 (0.935, 1.138)	0.538
BCd (μg/L)	0.780 (0.655, 0.928)	0.005*	0.910 (0.804, 1.031)	0.140
BCu (μg/L)	1.000 (0.999, 1.002)	0.793	0.999 (0.997, 1.002)	0.549
BPb (μg/L)	1.015 (0.997, 1.034)	0.103	0.994 (0.982, 1.007)	0.346
UMg (mg/g Cr)	0.999 (0.995, 1.004)	0.820	1.007 (0.998, 1.016)	0.127
UCa (mg/g Cr)	1.001 (0.998, 1.003)	0.696	0.997 (0.993, 1.001)	0.110
UFe (mg/g Cr)	1.009 (0.878, 1.161)	0.895	1.876 (1.290, 2.778)	0.001*
UZn (mg/g Cr)	0.915 (0.643, 1.301)	0.620	0.622 (0.465, 0.831)	0.001*
UAs (μg/g Cr)	1.005 (0.993, 1.017)	0.426	0.985 (0.963, 1.008)	0.204
UCd (μg/g Cr)	0.918 (0.774, 1.090)	0.329	1.047 (0.951, 1.153)	0.351
UCu (μg/g Cr)	1.001 (0.997, 1.006)	0.610	0.996 (0.981, 1.012)	0.655
UPb (μg/g Cr)	0.982 (0.919, 1.049)	0.587	1.010 (0.983, 1.038)	0.454

All the models have been adjusted for age, sex, body mass index, and smoking status. The normal fasting glucose group is set as the control, and the raw values of all variables are included in models. The total sample for the unpolluted area includes the normal fasting glucose group (n = 115) and the impaired fasting glucose group (n = 69) and that for the cadmium (Cd)-polluted area includes the normal fasting glucose group (n = 171) and the impaired fasting glucose group (n = 57). *P < 0.05. BAs, concentration of arsenic in the blood; BCa, concentration of calcium in the blood; BCd, concentration of cadmium in the blood; BCu, concentration of copper in the blood; BFe, concentration of iron in the blood; BMg, concentration of magnesium in the blood; BPb, concentration of lead in the blood; BZn, concentration of zinc in the blood; CI, confidence interval; OR, odds ratio; UAs, concentration of arsenic in the urine; UCa, concentration of calcium in the urine; UCd, concentration of cadmium in the urine; UCu, concentration of copper in the urine; UFe, concentration of iron in the urine; UMg, concentration of magnesium in the urine; UPb, concentration of lead in the urine; UZn, concentration of zinc in the urine.

mixture and FBG when the levels of all metals exceeded their 55th percentile (Figure 4a–d).

DISCUSSION

We evaluated the associations among mixed exposure to eight metals, diabetes mellitus prevalence, IFG rate and FBG levels in Cd-polluted and unpolluted areas in rural areas of southwest China. A separate analysis of the two areas showed that different metal exposure backgrounds might affect the associations between metal mixtures and FBG levels. UZn, UPb and UCu with high posterior inclusion probability in the BKMR analysis played major roles in the overall association between metal exposure and FBG levels in the unpolluted area, whereas BCd with high posterior inclusion probability dominated in the Cd-polluted area. For the unpolluted area, we found that BCd levels were negatively correlated with IFG rate and diabetes mellitus prevalence, whereas BFe and UPb levels were positively correlated with diabetes mellitus prevalence. Inverted U-shaped associations were found among UZn, UPb, UCu and FBG levels. For the Cd-polluted area, we found that BCd levels were negatively correlated with diabetes mellitus prevalence and FBG levels, and UZn levels were negatively correlated with IFG rate and diabetes mellitus prevalence. Meanwhile, UFe levels were positively correlated with IFG rate. We also found possible interactive effects between Zn and Pb, and between Cd and Ca on FBG levels.

A few prospective studies that explored the associations between metal exposure and diabetes mellitus presented the different roles of metals in diabetes mellitus development[15,17,25–30]. However, no unified conclusion about which metal is harmful, protective or irrelevant for diabetes mellitus has been achieved, partly because of the different metal mixture effects, implications of variable biological samples, times and levels of exposure in these studies.

The present results showed that BCd was negatively correlated with diabetes mellitus prevalence, IFG rate and FBG levels. This finding is consistent with those of Nakamura *et al.*[31] and Anetor *et al.*[32] However, it is inconsistent or even contrary to our initial assumption and the findings of other reports[15,33–35], due to two possible reasons. First, when humans or animals are exposed to low Cd concentrations for a short time, the secretory function of pancreatic islet cells is enhanced, resulting in low blood sugar. *In vitro* experiments have shown that the insulin release rate increases initially then decreases with the increase in exposure concentration and time when mice pancreatic islet cells are incubated with Cd^{2+}[36,37]. UCd is

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Table 8 | Logistic regression models for diabetes associated with multiple metals

	Unpolluted area (n = 135)		Cd-polluted area (n = 193)	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
BMg (mg/L)	0.968 (0.869, 1.077)	0.547	1.060 (0.957, 1.175)	0.266
BCa (mg/L)	1.005 (0.959, 1.052)	0.848	0.961 (0.904, 1.021)	0.197
BFe (mg/L)	1.008 (1.001, 1.014)	0.019*	1.006 (0.997, 1.014)	0.185
BZn (mg/L)	0.943 (0.704, 1.261)	0.691	0.765 (0.379, 1.546)	0.456
BAs (μg/L)	0.960 (0.902, 1.023)	0.209	0.959 (0.805, 1.141)	0.633
BCd (μg/L)	0.506 (0.288, 0.888)	0.018*	0.722 (0.536, 0.972)	0.032*
BCu (μg/L)	1.000 (0.997, 1.003)	0.976	1.001 (0.997, 1.005)	0.632
BPb (μg/L)	1.007 (0.952, 1.064)	0.818	0.986 (0.959, 1.014)	0.324
UMg (mg/g Cr)	1.004 (0.995, 1.014)	0.394	1.007 (0.995, 1.020)	0.260
UCa (mg/g Cr)	0.999 (0.992, 1.006)	0.777	1.000 (0.996, 1.005)	0.838
UFe (mg/g Cr)	0.714 (0.497, 1.028)	0.070	1.471 (0.931, 2.326)	0.098
UZn (mg/g Cr)	1.174 (0.830, 1.662)	0.364	0.609 (0.395, 0.939)	0.025*
UAs (μg/g Cr)	1.005 (0.977, 1.034)	0.724	0.983 (0.951, 1.016)	0.315
UCd (μg/g Cr)	0.851 (0.592, 1.225)	0.387	0.984 (0.793, 1.222)	0.886
UCu (μg/g Cr)	1.006 (0.997, 1.016)	0.192	1.005 (0.992, 1.019)	0.442
UPb (μg/g Cr)	1.185 (1.022, 1.376)	0.025*	0.983 (0.871, 1.109)	0.782

All the models have been adjusted for age, sex, body mass index and smoking status. The normal fasting glucose group is set as the control, and the raw values of all variables are included in models. The total sample for the unpolluted area includes the normal fasting glucose group (n = 115) and the diabetes mellitus group (n = 20) and that for the cadmium (Cd)-polluted area includes the normal fasting glucose group (n = 171) and the diabetes mellitus group (m = 22). *$P < 0.05$. BAs, concentration of arsenic in the blood; BCa, concentration of calcium in the blood; BCd, concentration of cadmium in the blood; BCu, concentration of copper in the blood; BFe, concentration of iron in the blood; BMg, concentration of magnesium in the blood; BPb, concentration of lead in the blood; BZn, concentration of zinc in the blood; CI, confidence interval; OR, odds ratio; UAs, concentration of arsenic in the urine; UCa, concentration of calcium in the urine; UCd, concentration of cadmium in the urine; UCu, concentration of copper in the urine; UFe, concentration of iron in the urine; UMg, concentration of magnesium in the urine; UPb, concentration of lead in the urine; UZn, concentration of zinc in the urine.

generally considered to reflect long-term Cd exposure and accumulation in the body, and BCd reflects recent exposure[38]. The Cd exposure levels of the present study population were lower than the World Health Organization's standards; that is, 10 μg /L for BCd and 5 μg/g Cr for UCd[39]. These findings suggest that relatively short-term, low-level Cd exposure might be related to temporary low FBG levels. Second, co-exposure to metals might confound the real effects of Cd on blood sugar. For example, we found that the BZn levels among the present study population were positively correlated with BCd levels in Spearman's rank correlation. Zn and Cd, belonging to the same column in the periodic table, compete for the same biological targets in the pathological process of diabetes due to their shared binding sites and/or ligands[40-43]. Furthermore, the BKMR models suggested a potential interaction effect between BCd and BCa on FBG levels in the Cd-polluted area. Ca might interfere with the effects of Cd on FBG by mediating signal transduction during insulin secretion[44]. Another study based on the relationship between multi-metal exposure and FBG levels showed that the relationship between Cd and FBG might be affected by other co-exposed metals[45]. Overall, the real effect of Cd on diabetes mellitus and blood sugar still needs to be studied using enlarged samples, and the co-exposure of other metals should be considered.

Fe overload is a known risk factor for type 2 diabetes mellitus and thought to be involved in the insulin secretion mechanism. High Fe levels can generate free radicals and reactive oxygen species. Increased and continuous exposure to intracellular reactive oxygen species can cause insulin secretion disorders[46]. Many studies have shown that excessive Fe is associated with elevated FBG levels and increased diabetes mellitus prevalence. For example, Blesia *et al.*[47] showed that pancreatic β-cells are susceptible to excessive Fe accumulation, leading to decreased cell viability and reduced insulin secretion[47]. A study that used Mendelian randomization found a causal relationship between increased systemic Fe status and increased prevalence of type 2 diabetes mellitus by analyzing serum Fe, ferritin, transferrin and transferrin saturation levels in 74,124 type 2 diabetes mellitus cases and 824,006 controls[48]. Furthermore, many studies have found that the higher the Fe exposure level is in women during pregnancy, the higher the prevalence of gestational diabetes mellitus is[49-53]. These findings are consistent with the present results that Fe exposure level is positively correlated with IFG rate and diabetes mellitus prevalence.

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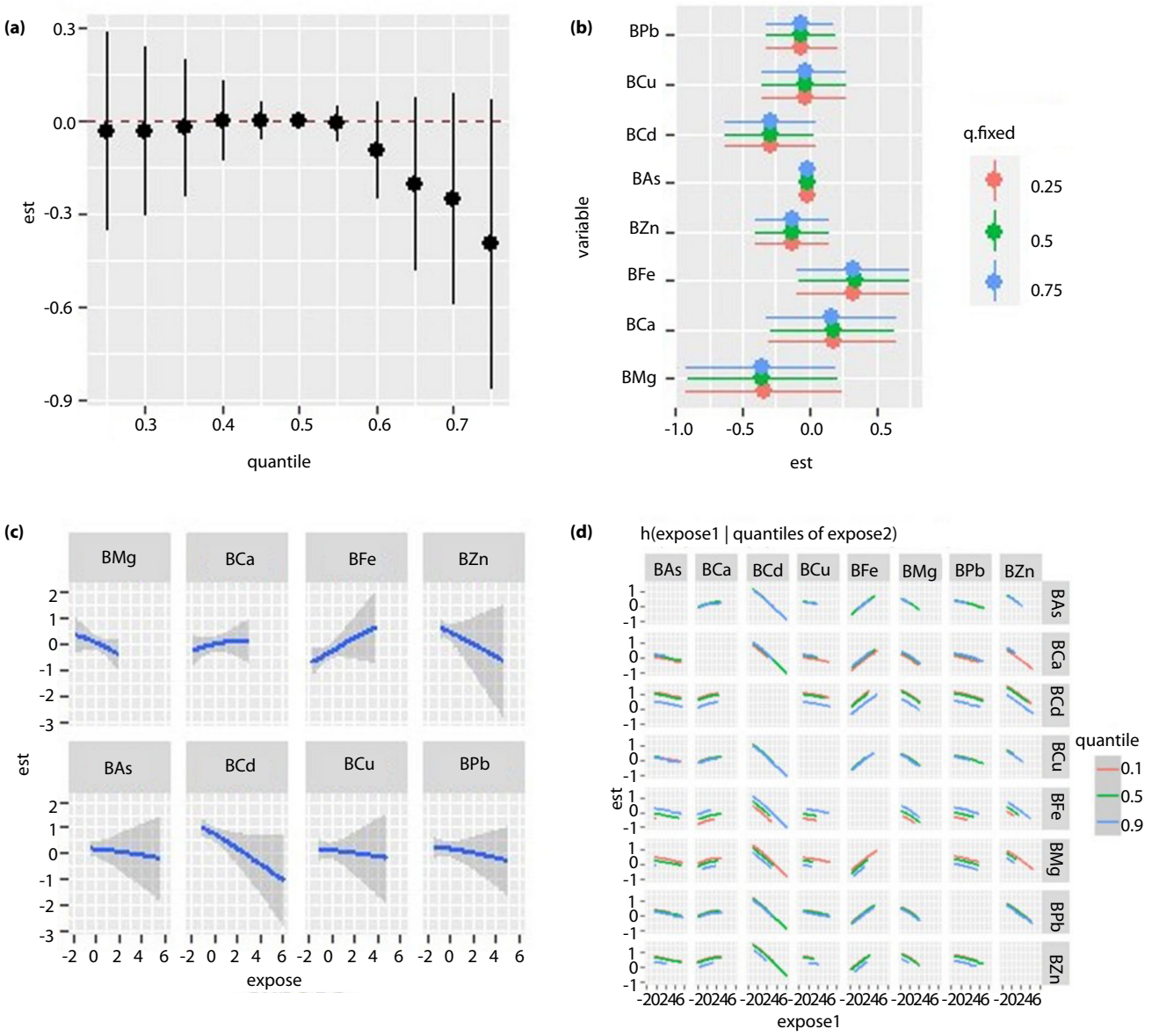

Figure 1 | Regression analysis between blood metal and fasting blood glucose (FBG) levels in the unpolluted area. All the models have been adjusted for age, sex, body mass index and smoking status. 'Est' stands for 'estimate,' which means that the estimates of the effects include the overall, individual and interactive effects of different metals on FBG when the metal levels change. 'Expose' stands for metal exposure levels. Here, we use the z-score for all the exposures to have the same scale. (a) Overall effects of mixed-metal exposure: the variation of FBG when all the metals are at a particular quantile compared with when all of them are at their median value. (b) Contribution of single-metal exposure to the overall effects: the variation of FBG when a single metal is at the 75th percentile compared with when it is at its 25th percentile, and all of the remaining metals are fixed at either the 25th, 50th or 75th percentile. (c) Univariate exposure–response function: the univariate relationship between each metal and FBG when all of the other metals are fixed at the 50th percentile. (d) Bivariate exposure–response function: the exposure-response function of a single metal when the second metal is fixed at either the 10th, 50th or 90th percentile and the remaining metals are fixed to a particular value. BAs, concentration of arsenic in the blood; BCa, concentration of calcium in the blood; BCd, concentration of cadmium in the blood; BCu, concentration of copper in the blood; BFe, concentration of iron in the blood; BMg, concentration of magnesium in the blood; BPb, concentration of lead in the blood; BZn, concentration of zinc in the blood.

Cu and Zn are essential trace elements for humans. Cu can bind to superoxide dismutase or interact with metallothioneins to clear free radicals efficiently, thereby protecting pancreatic β-cells from damage or apoptosis caused by oxidative stress[54,55]. Zn is an anti-oxidant and can directly participate in the synthesis, storage and secretion of insulin in pancreatic β-cells[6].

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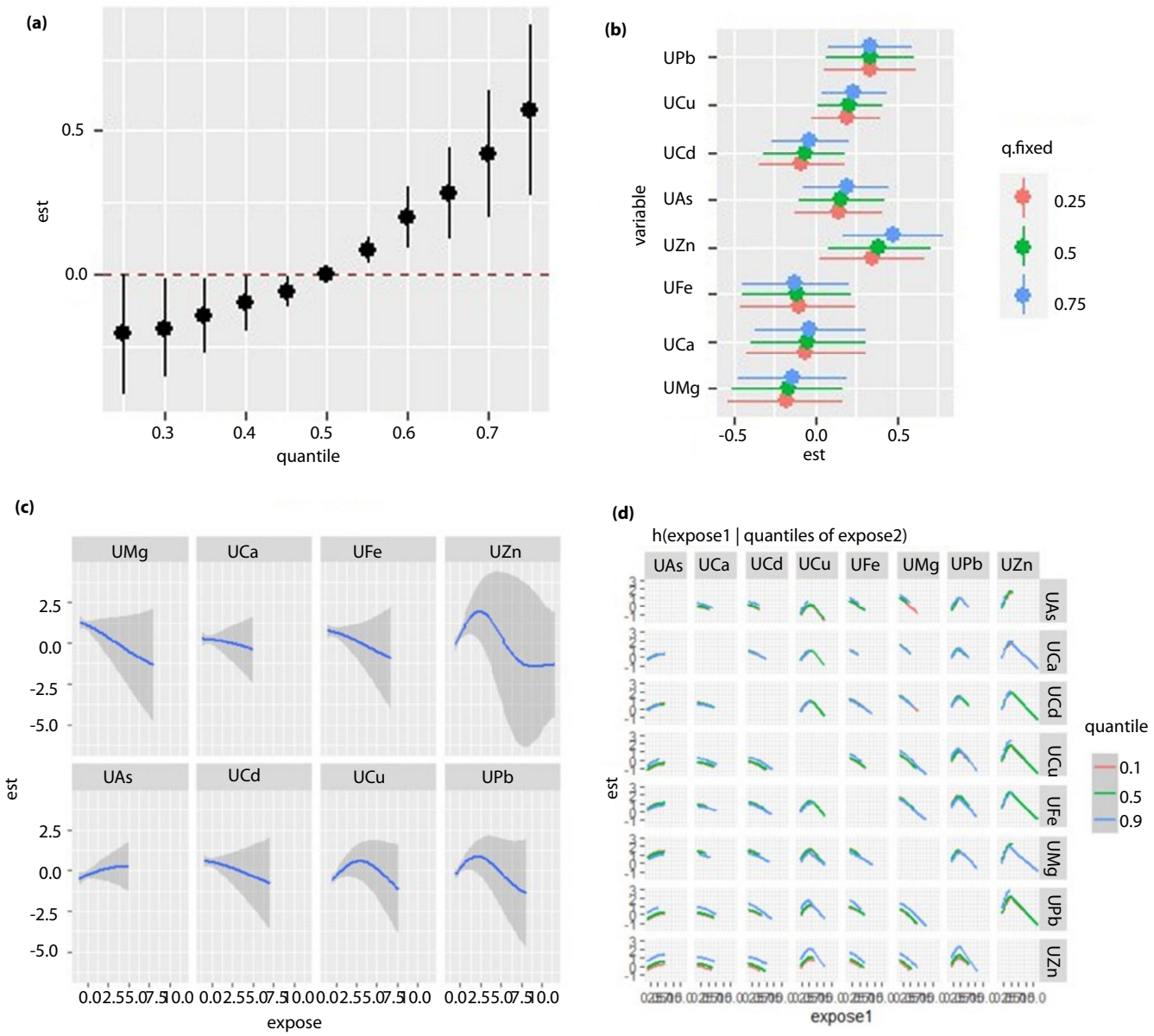

Figure 2 | Regression analysis between urine metal and fasting blood glucose (FBG) levels in the unpolluted area. All of the models have been adjusted for age, sex, body mass index and smoking status. 'Est' stands for 'estimate', which means that the estimates of the effects include the overall, individual and interactive effects of different metals on FBG when the metal levels change. 'Expose' stands for metal exposure levels. Here, we use the z-score for all the exposures to have the same scale. (a) Overall effects of mixed-metal exposure: the variation of FBG when all the metals are at a particular quantile compared with when all of them are at their median value. (b) Contribution of single-metal exposure to the overall effects: the variation of FBG when a single metal is at the 75th percentile compared with when it is at its 25th percentile, and all of the remaining metals are fixed at either the 25th, 50th, or 75th percentile. (c) Univariate exposure–response function: the univariate relationship between each metal and FBG when all of the other metals are fixed at the 50th percentile. (d) Bivariate exposure–response function: the exposure-response function of a single metal when the second metal is fixed at either the 10th, 50th or 90th percentile and the remaining metals are fixed to a particular value. UAs, concentration of arsenic in the urine; UCa, concentration of calcium in the urine; UCd, concentration of cadmium in the urine; UCu, concentration of copper in the urine; UFe, concentration of iron in the urine; UMg, concentration of magnesium in the urine; UPb, concentration of lead in the urine; UZn, concentration of zinc in the urine.

People with Zn deficiency are likely to develop insulin resistance. Zn supplementation can alleviate insulin resistance, which is conducive to blood sugar control in people with and without diabetes mellitus[56-58]. Therefore, Cu and Zn are beneficial for decreasing blood sugar levels, and reducing the IFG rate and diabetes mellitus prevalence to a certain extent, which is

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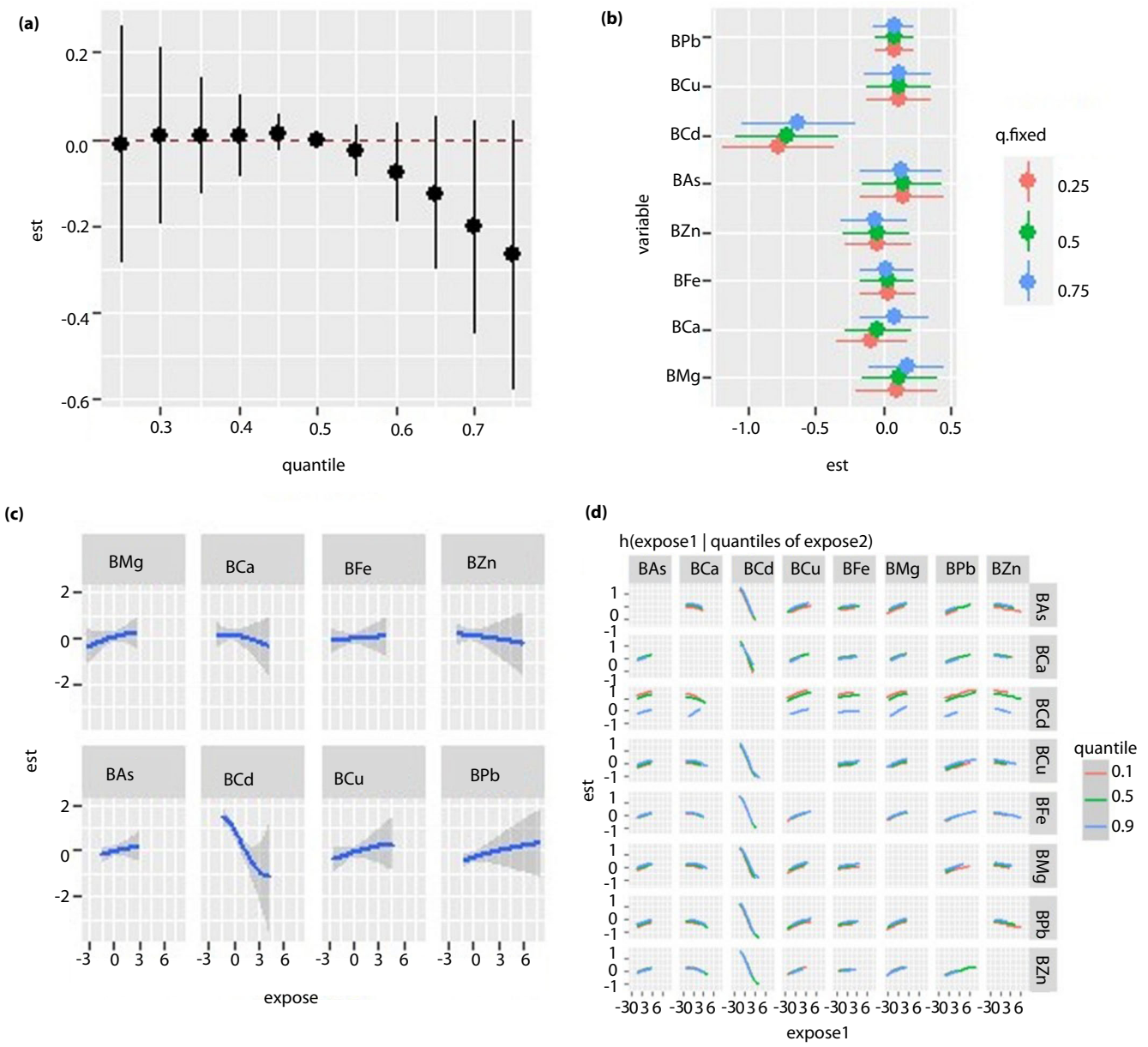

Figure 3 | Regression analysis between blood metal and fasting blood glucose (FBG) levels in the cadmium-polluted area. All the models have been adjusted for age, sex, body mass index and smoking status. 'Est' stands for 'estimate', which means that the estimates of the effects include the overall, individual and interactive effects of different metals on FBG when the metal levels change. 'Expose' stands for metal exposure levels. Here, we use the z-score for all the exposures to have the same scale. (a) Overall effects of mixed-metal exposure: the variation of FBG when all the metals are at a particular quantile compared with when all of them are at their median value. (b) Contribution of single-metal exposure to the overall effects: the variation of FBG when a single metal is at the 75th percentile compared with when it is at its 25th percentile, and all of the remaining metals are fixed at either the 25th, 50th or 75th percentile. (c) Univariate exposure–response function: the univariate relationship between each metal and FBG when all of the other metals are fixed at the 50th percentile. (d) Bivariate exposure–response function: the exposure–response function of a single metal when the second metal is fixed at either the 10th, 50th or 90th percentile and the remaining metals are fixed to a particular value. BAs, concentration of arsenic in the blood; BCa, concentration of calcium in the blood; BCd, concentration of cadmium in the blood; BCu, concentration of copper in the blood; BFe, concentration of iron in the blood; BMg, concentration of magnesium in the blood; BPb, concentration of lead in the blood; BZn, concentration of zinc in the blood.

consistent with our conclusion that UZn has a negative correlation with IFG rate and diabetes mellitus prevalence. However, Cu and Zn also have pro-oxidant effects. Cu can regulate electron transfer, and increased Zn levels can inhibit metabolism and the mitochondrial function, thus promoting the production of reactive oxygen species[59,60]. The results of a

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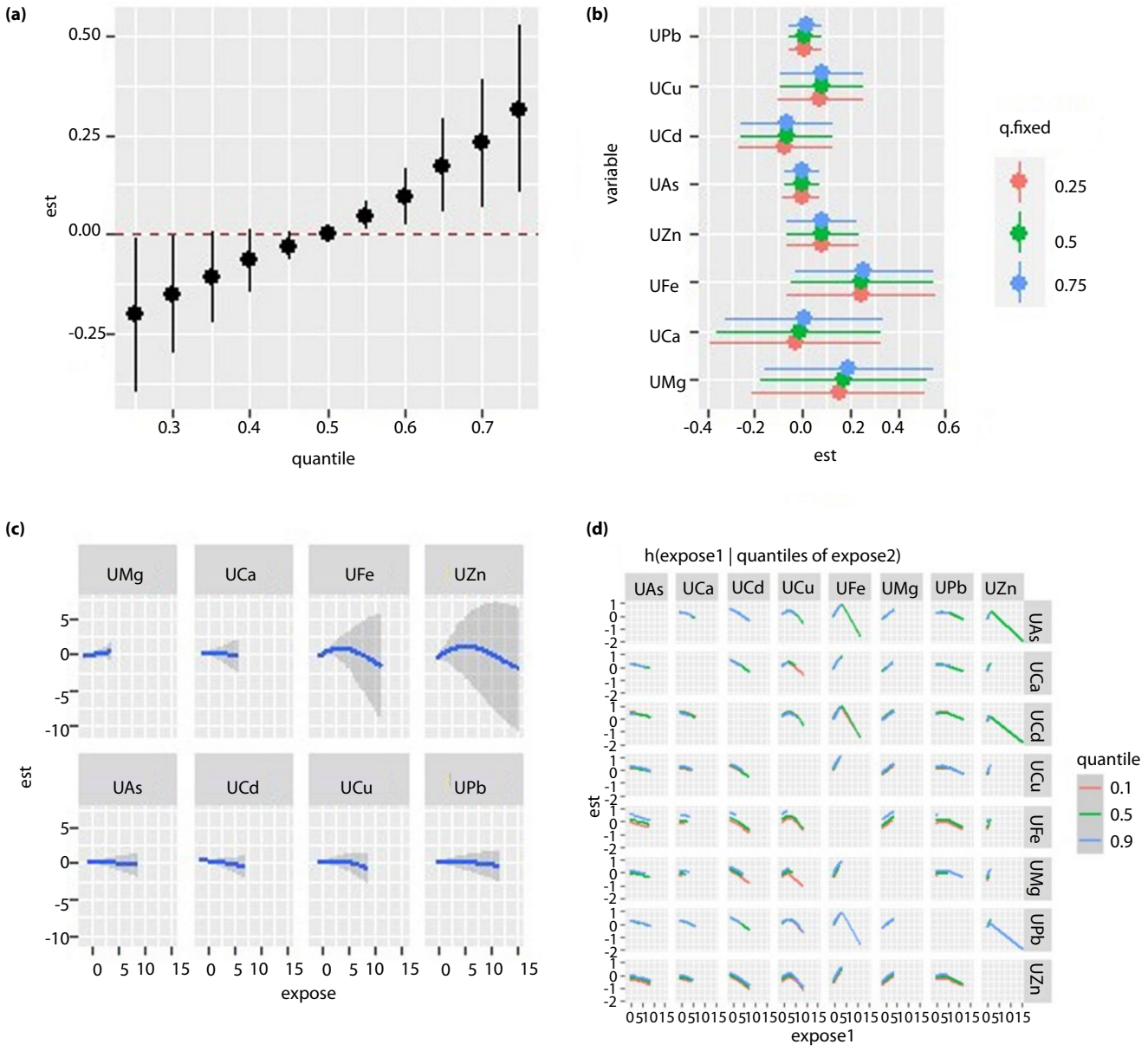

Figure 4 | Regression analysis between urine metal and fasting blood glucose (FBG) levels in the cadmium-polluted area. All the models have been adjusted for age, sex, body mass index and smoking status. 'Est' stands for 'estimate', which means that the estimates of the effects include the overall, individual and interactive effects of different metals on FBG when the metal levels change. 'Expose' stands for metal exposure levels. Here, we use the z-score for all the exposures to have the same scale. (a) Overall effects of mixed-metal exposure: the variation of FBG when all the metals are at a particular quantile compared with when all of them are at their median value. (b) Contribution of single-metal exposure to the overall effects: the variation of FBG when a single metal is at the 75th percentile compared with when it is at its 25th percentile, and all of the remaining metals are fixed at either the 25th, 50th or 75th percentile. (c) Univariate exposure–response function: the univariate relationship between each metal and FBG when all of the other metals are fixed at the 50th percentile. (d) Bivariate exposure-response function: the exposure-response function of a single metal when the second metal is fixed at either the 10th, 50th or 90th percentile and the remaining metals are fixed to a particular value. UAs, concentration of arsenic in the urine; UCa, concentration of calcium in the urine; UCd, concentration of cadmium in the urine; UCu, concentration of copper in the urine; UFe, concentration of iron in the urine; UMg, concentration of magnesium in the urine; UPb, concentration of lead in the urine; UZn, concentration of zinc in the urine.

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multisite, multiethnic cohort study on women at midlife also showed that women with excess zinc in their urine might face an elevated risk of diabetes[15]. The mechanism might be related to the loss of zinc in pancreatic β-cells, which leads to a decrease in insulin secretion[15,61]. This dual effect might be one of the reasons for the inverted U-shaped exposure–response relationship between Zn and Cu and FBG levels.

Pb is a common environmental toxic metal[62]. A few studies investigated the relationship between Pb exposure and diabetes mellitus prevalence, and suggested that Pb exposure might promote the occurrence and development of diabetes mellitus[63]. A possible mechanism is that Pb can activate the expression of genes related to glucose metabolism, such as phosphoenolpyruvate carboxykinase, glucose-6-phosphatase and glycogen synthase kinase-3 beta, thereby increasing the activity of hepatic gluconeogenesis enzymes and interfering with the insulin secretory function, and finally leading to increased blood sugar[64,65]. However, other studies did not find a relationship between Pb and diabetes mellitus[16,17,19]. We discovered in the present study that Pb exerted a blood sugar-lowering effect when it rose to a certain level, which might be related to the co-exposure of Pb and other metals; however, the specific reason is unclear.

We also found that UZn and UPb exerted a positive interactive effect on FBG. However, many previous studies supported the conclusion that a negative correlation exists between Zn and Pb, and exerts an antagonistic effect on the body[66]. We speculate that this might be due to the influence of other metal elements covering up the real association between Zn and Pb, or our relatively small sample size and low Pb level led to a false connection. Given this limited and conflicting epidemiological evidence, and the high variability and heterogeneity of Zn, Pb and Cu exposure levels in different studies, further research is required to clarify the real individual and interactive effects of Cu, Zn, and Pb on diabetes mellitus and blood sugar.

The present study had the following advantages. First, it analyzed the relationship between metal levels in blood and urine and diabetes mellitus, thereby making up for the shortcomings of using only a kind of biological sample and improving the sensitivity of the research results. Second, we evaluated the effects of metal mixtures, a single metal and metal-to-metal interactions on FBG when exposed to multiple metals, and analyzed the exposure–response relationship between each metal and FBG by using the BKMR model. Finally, the present study is the first to explore the relationship between multi-metal exposure and diabetes mellitus in rural areas in southwest China. It can serve as preliminary evidence for the effects of multi-metal exposure on diabetes mellitus in the population in this region.

However, the present research also had limitations. First, due to the limits of cross-sectional studies, we could not establish a causal relationship between metal exposure and diabetes mellitus or FBG. Second, we adopted only FBG as a diagnostic indicator of diabetes mellitus, making the determination of diabetes mellitus cases not accurate enough. Other indicators, such as glycosylated hemoglobin and oral glucose tolerance test, should be included in the future. Third, the occurrence and development of diabetes are affected by many factors, including genetics, lifestyle, personal characteristics and environment. We did not adjust for all possible potential confounding factors. Fourth, excluding individuals taking medications might create selection bias, and the sample size of the present study was smaller than calculated. More samples would make the results more reliable.

In summary, the present findings suggest that multi-metal exposure, especially Cd, Fe, Zn, Cu and Pb, is linked to diabetes mellitus. For instance, diabetes mellitus prevalence and IFG rate were negatively correlated with Cd and Zn levels, but positively correlated with Fe levels. Non-linear exposure–response relationships were also observed between Zn, Pb, and Cu and FBG levels. Furthermore, interactive effects existed between Zn and Pb, and between Cd and Ca on FBG levels. The underlying mechanisms regarding such an interaction are perhaps related to similar biotransportation ways, signaling pathways or other competitive/synergistic action of metals. Future research should explore the effects and mechanisms of metal-to-metal interactions on the etiology of diabetes mellitus.

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DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: The protocol for this study was reviewed and approved by the Ethical Committee of West China School of Public Health and West China Fourth Hospital, Sichuan University (Approval No. Gwll2013052).

Informed consent: All survey participants signed informed consent.

Approval date of Registry and the Registration No. of the study/trial: N/A.

Animal studies: N/A.

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