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Exploring the Interactions of Urinary Metals and the Mediating Role of Oxidative Stress in Parkinson's Disease Risk: An Epidemiological Study in the Elderly

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Reagent and materials

The mixed metal standard solution (1000 mg L^{-1}) containing manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), cobalt (Co), selenium (Se), chromium (Cr), nickel (Ni), cadmium (Cd), and lead (Pb) was purchased from Agilent Technologies (Santa Clara, CA, USA). 8-OHdG and its internal standard, 8-OHdG-15N5 (25 mg L^{-1}) were purchased from Sigma-Aldrich (Darmstadt, Germany). Methanol (chromatographic grade, ≥ 99.0%), acetonitrile (chromatographic grade, ≥ 99.0%), and β-glucuronidase (> 100000 units mL^{-1}) were obtained from CNW (Shanghai, China). Ammonium acetate (≥ 98.0%) was purchased from Macklin (Shanghai, China). Nitric acid (65%) was also purchased from Sigma-Aldrich (Darmstadt, Germany). Deionized water was prepared using a water purification system (Millipore, Billerica, MA, USA).

Sample preparation

Urine samples were taken out from the -80℃ refrigerator and thawed in the 4 ℃ refrigerator. After complete thawing, urine samples were mixed upside down twice. Then 800 μL of urine sample was transferred to a plastic tube and centrifuged at 8000 rpm for 5 min. Subsequently, 0.5 mL of supernatant was transferred to a 15 mL plastic tube, added with 9.5 mL of 1% HNO_3, and subjected to instrumental analysis.

The preparation of urinary 8-OHdG was according to a procedure described previously (Fu et al., 2024). After the urine sample was pre-thawed, take 1 mL of urine into a labeled glass tube with a lid. A mixed internal standard solution of 40 μL with a concentration of 100 μg L^{-1} was vertically added to the sample, followed by 1 mL ammonium acetate buffer solution, and 20 μL β-glucuronidase was added to the sample. The sample was subjected to enzymatic hydrolysis at 37 ℃ and a dark water bath for 12-16 h. The HC-C18 solid phase extraction column was installed on the

solid-phase extraction device. 5 mL methanol and 10 mL ultra-pure water were added successively to activate the column. After the activation was completed, the sample was poured into the column, and the glass tube was rinsed with 1 mL 5% methanol aqueous solution. After vortex shaking, the rinsing solution was poured into the column. After the liquid is completely drained, turn on the vacuum pump and run for 1 min to ensure that the solid phase extraction column is completely drained. The corresponding labeled glass test tube was placed in the solid phase extraction device to accept the eluent, and the sample was added to the 4 mL acetonitrile elution column. After the acetonitrile dried naturally, the sample was vacuumed again for 1 min. The test tube containing the eluent was installed on the nitrogen blower, and the nitrogen was concentrated until it was nearly dry. 400 μL of 60% methanol aqueous solution was added into the glass tube for redissolution. After vortex shock, the filtered samples were transferred to the sample vial equipped with a glass-lined tube and stored in the refrigerator at -20 ℃ for detection.

Table S1 Instrumental parameters of ICP-MS

Parameters	Value
Plasma radio frequency power	1500 W
Plasma gas flow rate	15 L min^{-1}
Carrier gas	High purity argon gas (99.999%)
Carrier gas flow rate	0.10 L min^{-1}
Auxiliary air velocity	0.10 L min^{-1}
Atomizing pump	0.10 rps
S/C temperature	2℃
Depth of sampling	8.0 mm
resolution	0.6—0.7 amu
Number of scans	3
Helium flow rate in the collision cell	5.0 L min^{-1}
Pattern	KED Pattern

Table S2 Information on the mass spectra of 8-OHdG

Classification	Name of standard	Q1 (DA)	Q3 (DA)	DP (V)	CE (V)	Retention time (msec)
Markers of	8-OHdG	282.2	192.0	-144.0	-18.0	25.0
oxidative stress	8-OHdG-$^{15}N_5$	287.0	197.0	-105.0	-25.0	25.0

DP: Declustering potential; CE: Collision energy.

Table S3 Liquid phase gradient elution procedure for 8-OHdG detection

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.00	95.0	5.00
1.00	80.0	20.0
12.0	5.00	95.0
13.0	95.0	5.00
18.0	95.0	5.00

Mobile phase A: ultrapure water; Mobile phase B: pure methanol.

Table S4 Evaluation of urinary heavy metals and 8-OHdG detection methods and quality control indicators

Substances	Standard curve	R^2	LOD (μg L^{-1})	LOQ (μg L^{-1})	Standard recovery (%)
Mn	y = 1.187x–0.042	0.999	0.043	0.128	107
Fe	y = 1.962x+4.632	0.999	0.391	1.172	99.0
Cu	y = 4.435x+1.231	0.999	0.054	0.162	95.4
Zn	y = 0.712x–0.088	0.999	0.066	0.197	102
Co	y = 5.727x+0.004	0.999	0.003	0.008	100
Se	y = 0.008x–0.001	0.999	0.436	1.307	118
Cr	y = 2.583x+1.332	0.999	0.009	0.027	97.2
Ni	y = 1.649x+0.013	0.999	0.018	0.054	99.3
Cd	y = 0.109x–0.00008	0.999	0.00005	0.0001	101
Sn	y = 0.075x+0.002	0.999	0.022	0.067	106
Pb	y = 0.189x+0.005	0.999	0.018	0.054	112
8-OHdG	y = 18411x+492	0.999	0.056	0.167	76.4

R^2: Standard curve correlation coefficient.

Reference

Fu, J., Yao, Y., Huang, Z., Guo, Z., Chen, X., Tang, X., Ge, Y., Xiao, Q., Sha, Y., Lu, S., 2024. Sex-Specific and Trimester-Specific Associations of Prenatal Exposure to Bisphenols, Parabens, and Triclosan with Neonatal Birth Size and Gestational Age. Environ. Sci. Technol. 58, 13687–13696. https://doi.org/10.1021/acs.est.4c04940