SUPPLEMENTAL MATERIAL

Circulating multiple metals and incident stroke in Chinese adults: the Dongfeng-Tongji cohort

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Supplemental Methods

Confirmation of incident stroke cases

Stroke was diagnosed following the WHO criteria as a constellation of neurologic deficits of sudden or rapid onset that lasted at least 24 hours or until death with no apparent cause other than that of vascular origin.[1] The follow-up questionnaires recorded the information of history of chronic disease. The DMC health-care service system covered all the participants and provided complete morbidity and mortality records until December 31, 2016. Possible stroke cases were initially identified through follow-up questionnaires, medical insurance documents, death certificates and hospital records. An expert panel of physicians reviewed medical records and adjudicated definite stroke cases according to clinical symptoms and confirmatory imaging findings. Moreover, fatal stroke cases were identified by death certificates with International Classification of Diseases (ICD) codes (ICD-10 I60, I61, I63, I64 and ICD-10 I69.0, I69.1, I69.3, I69.4). The researchers crosschecked all the dates of stroke events through medical records and death certificates. Incident stroke cases were identified as the first occurrence of definite stroke between the sampling date and December 31, 2016. All the definite stroke cases included in our analysis received CT or MRI examination. Among the 1,304 stroke cases, a total of 619 subjects received CT and 411 received MRI, and the other 274 participants underwent both CT and MRI.

Covariate data collection

Trained interviewers used a semi-structured questionnaire to collect the information of socio-demographics (e.g., age and sex), lifestyle habits (e.g., smoking status, drinking status and physical activity), personal and family history of chronic diseases (e.g., stroke, CHD, cancer, hypertension, hyperlipidemia and diabetes mellitus) and medication use. Smokers were classified as current smokers who smoked at least one cigarette per day over the last six months, ever smokers who quitted smoking for more than one month, and never smokers. Drinkers were classified as current drinkers who drunk more than once per week over the last six months, ever drinkers who quitted drinking for more than one month, and never drinkers. Regular exercise was defined as physical exercise for at least 30 min in no less than 5 days per week for more than half a year.

Physical examinations including measurements of weight, height, blood pressures (BPs), blood lipids and fasting glucose were taken by qualified physicians in Dongfeng General Hospital. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters and overweight was defined as BMI≥24 kg/m^2. Hyperlipidemia was defined as self-reported hyperlipidemia, or using anti-hyperlipidemia medications, or total cholesterol (TC)≥6.22 mmol/L, or triglycerides (TG)≥2.26 mmol/L, or high-density lipoprotein cholesterol (HDL-C)<1.04 mmol/L, or high-density lipoprotein cholesterol (LDL-C)≥4.14 mmol/L. Diabetes was defined as self-reported diabetes mellitus, or use of anti-diabetic medications, or fasting glucose≥7.0 mmol/L. Hypertension was defined as self-reported hypertension, using antihypertensive medication, or having systolic blood pressure (SBP)≥140 mmHg or diastolic blood pressure (DBP)≥90 mmHg.

Quality control methods for metal measurement

Matched case-control sets were assessed in the same analytical run but in random order with laboratory personnel blinded to the case-control status. The methods of accuracy evaluation and quality control have been described previously.[2] We assayed a spiked pooled specimen of 100 randomly selected plasma samples for the metals without certified reference samples (rubidium, mercury, titanium and tungsten). The spike recovery values of those four metals ranged from 84.40% to 104.07%. Intra-assay and inter-assay coefficients of variations for all plasma metals were below 15% (Table I). The limits of detection (LOD) for plasma metals were presented in Table I. For participants below the LOD, the metal concentrations were substituted with the value half of the detection limit.

Table I. Limits of detection, percentages of samples below detection limits, intra-assay and inter-assay coefficients of variation (total n=2608)

Plasma metals	LOD (μg/L)	Total No. (%) <LOD*	Ischemic stroke No. (%) <LOD*	Hemorrhagic stroke No. (%) <LOD*	Intra-assay CV%	Inter-assay CV%
Aluminum	0.4267	56 (2.15)	48 (2.32)	8 (1.49)	6.00	6.86
Antimony	0.0019	606 (23.24)	472 (22.80)	134 (24.91)	7.07	4.60
Arsenic	0.0141	6 (0.23)	5 (0.24)	1 (0.19)	3.60	5.37
Barium	0.0347	0 (0.00)	0 (0.00)	0 (0.00)	4.09	4.44
Cadmium	0.0015	15 (0.58)	14 (0.68)	1 (0.19)	5.39	6.33
Chromium	0.0680	2 (0.08)	2 (0.10)	0 (0.00)	5.74	5.90
Cobalt	0.0033	24 (0.92)	21 (1.01)	3 (0.56)	5.02	4.55
Copper	0.0128	0 (0.00)	0 (0.00)	0 (0.00)	4.29	4.98
Iron	0.5879	0 (0.00)	0 (0.00)	0 (0.00)	5.26	6.23
Lead	0.0114	0 (0.00)	0 (0.00)	0 (0.00)	9.92	4.75
Manganese	0.0169	9 (0.35)	8 (0.39)	1 (0.19)	6.49	4.66
Mercury	0.0050	42 (1.61)	34 (1.64)	8 (1.49)	4.38	8.32
Molybdenum	0.0042	0 (0.00)	0 (0.00)	0 (0.00)	7.67	5.51
Nickel	0.0382	61 (2.34)	41 (1.98)	20 (3.72)	6.27	5.70
Rubidium	0.0075	0 (0.00)	0 (0.00)	0 (0.00)	3.69	5.33
Selenium	0.0323	0 (0.00)	0 (0.00)	0 (0.00)	5.05	8.53
Strontium	0.0228	0 (0.00)	0 (0.00)	0 (0.00)	4.34	5.61
Thallium	0.0003	0 (0.00)	0 (0.00)	0 (0.00)	4.83	4.25
Tin	0.0100	1368 (52.45)	1048 (50.63)	320 (59.48)	9.38	5.93
Titanium	0.1527	0 (0.00)	0 (0.00)	0 (0.00)	5.11	9.53
Tungsten	0.0010	222 (8.51)	177 (8.55)	45 (8.36)	12.43	6.78
Uranium	0.0004	567 (21.74)	434 (20.97)	133 (24.72)	13.14	11.72
Vanadium	0.0043	0 (0.00)	0 (0.00)	0 (0.00)	4.57	3.46
Zinc	0.7324	0 (0.00)	0 (0.00)	0 (0.00)	3.67	5.45

Abbreviations: LOD, limit of detection; CV, coefficient of variation.
*Number and percentage of samples below LOD.

Table II. Basic characteristics and concentrations of plasma metals of study participants at baseline

Variables	Ischemic stroke			Hemorrhagic stroke		
	Controls (n=1035)	Cases (n=1035)	*P* value*	Controls (n=269)	Cases (n=269)	*P* value*
Age (year)	66.8±7.5	66.8±7.6	0.79	65.62±7.7	65.62±7.7	1.00
Male, No. (%)	653 (63.1)	653 (63.1)	1.00	157 (58.4)	157 (58.4)	1.00
Body mass index (kg/m^2)	24.4±3.0	24.7±3.2	0.019	24.2±3.1	24.4±3.5	0.44
Smoking status, No. (%)			0.08			0.63
Current smoker	251 (24.2)	296 (28.6)		63 (23.4)	65 (24.2)	
Former smoker	152 (14.7)	142 (13.7)		34 (12.7)	41 (15.2)	
Never smoker	632 (61.1)	597 (57.7)		172 (63.9)	163 (60.6)	
Drinking status, No. (%)			0.21			0.48
Current drinker	277 (26.8)	310 (30.0)		62 (23.1)	72 (26.8)	
Former drinker	72 (6.9)	61 (5.9)		14 (5.2)	17 (6.3)	
Never drinker	686 (66.3)	664 (64.1)		193 (71.7)	180 (66.9)	
Regular exercise, No. (%)†	761 (73.5)	735 (71.0)	0.20	202 (75.1)	196 (72.9)	0.56
Family history of stroke, No. (%)	46 (4.4)	29 (2.8)	0.046	14 (5.2)	7 (2.6)	0.12
Hyperlipidemia, No. (%)‡	443 (42.8)	485 (46.9)	0.06	129 (48.0)	134 (49.8)	0.67
Diabetes, No. (%)§	186 (18.0)	274 (26.5)	<0.001	51 (19.0)	63 (23.4)	0.21
Hypertension, No. (%)‖	568 (54.9)	725 (70.1)	<0.001	158 (58.7)	200 (74.4)	<0.001
Plasma metals (μg/L)						
Aluminum	56.78 (36.96-103.64)	57.13 (37.51-114.25)	0.50	60.23 (38.98-131.83)	56.84 (36.65-97.21)	0.17
Arsenic	1.86 (1.09-3.77)	1.95 (1.06-4.07)	0.34	1.91 (1.04-4.38)	1.83 (1.03-4.07)	0.20
Barium	38.33 (23.04-67.81)	40.57 (23.62-70.73)	0.20	41.37 (23.12-69.01)	37.16 (22.05-72.71)	1.00
Cobalt	0.17 (0.14-0.21)	0.17 (0.13-0.21)	0.99	0.16 (0.13-0.21)	0.17 (0.13-0.22)	0.91
Copper	953.14 (850.30-1071.60)	979.48 (875.80-1112.71)	<0.001	999.38 (884.52-1120.40)	1003.70 (883.30-1115.17)	0.68
Lead	13.30 (8.75-23.75)	14.34 (9.22-25.67)	0.043	13.18 (8.79-23.70)	14.37 (9.02-26.16)	0.91
Manganese	2.79 (2.12-3.90)	2.87 (2.10-3.97)	0.97	2.97 (2.06-4.05)	2.76 (2.03-3.85)	0.28

Mercury	0.55 (0.36-0.84)	0.53 (0.35-0.78)	0.47	0.55 (0.33-0.86)	0.51 (0.32-0.77)	0.046
Molybdenum	1.27 (1.00-1.66)	1.29 (1.02-1.74)	0.003	1.34 (1.02-1.71)	1.34 (1.03-1.83)	0.04
Nickel	2.44 (1.83-3.55)	2.49 (1.84-3.64)	0.11	2.36 (1.77-3.36)	2.29 (1.64-3.08)	0.24
Rubidium	348.50 (309.66-394.25)	345.22 (308.26-390.88)	0.56	357.90 (318.82-396.32)	348.31 (298.94-384.20)	0.009
Selenium	66.36 (57.50-78.59)	66.02 (55.91-77.15)	0.027	68.86 (57.14-78.66)	63.85 (55.37-76.20)	0.03
Strontium	33.92 (28.56-41.63)	34.86 (29.23-41.96)	0.42	34.59 (28.30-40.55)	36.08 (29.64-43.44)	0.038
Thallium	0.10 (0.08-0.13)	0.10 (0.08-0.14)	0.32	0.10 (0.08-0.14)	0.10 (0.08-0.13)	0.12
Titanium	49.09 (41.49-56.95)	50.23 (42.41-58.13)	0.07	44.13 (35.50-55.49)	43.35 (34.24-55.25)	0.82
Tungsten	0.05 (0.03-0.07)	0.05 (0.04-0.07)	0.55	0.05 (0.03-0.07)	0.05 (0.03-0.07)	0.73
Vanadium	1.41 (1.13-1.95)	1.43 (1.14-1.98)	0.81	1.42 (1.14-2.01)	1.41 (1.12-1.93)	0.76
Zinc	1383.21 (1046.25-2775.78)	1454.18 (1045.67-3399.51)	0.31	1461.55 (1072.69-2830.71)	1432.18 (1027.95-2742.95)	0.77

Normally distributed variables were presented as mean±SD or median (25th–75th percentiles). Categorical variables were presented as numbers (percentage). Plasma antimony, tin and uranium with low detection rates were excluded from further analyses because of many samples below the limits of detection (23.24%, 52.45% and 21.74%, respectively). Plasma cadmium, chromium, and iron were excluded from further analyses because of concerns about the use of plasma concentrations as biomarkers.

*P values were derived from Student's t tests or Mann-Whitney U tests for continuous variables according to the data distribution, as well as Chi-square tests for the category variables.

†Regular exercise was defined as physical exercise for at least 30 min in no less than 5 days per week for more than half a year.

‡Hyperlipidemia was defined as self-reported hyperlipidemia, or using anti-hyperlipidemia medications, or total cholesterol (TC)≥6.22 mmol/L, or triglycerides (TG)≥2.26 mmol/L, or high-density lipoprotein cholesterol (HDL-C)<1.04 mmol/L, or high-density lipoprotein cholesterol (LDL-C)≥4.14 mmol/L.

§Diabetes was defined as self-reported diabetes mellitus, or use of anti-diabetic medications, or fasting glucose≥7.0 mmol/L.

‖Hypertension was defined as self-reported hypertension, using antihypertensive medication, or having systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP)≥90 mmHg.

Table III. Adjusted odds ratios (95% CI) for incident stroke according to plasma metals

Plasma metals	Tertiles of plasma metals (μg/L)			*P*-trend	*FDR*[*]	Linear model[†]
	T1	T2	T3			
Ischemic stroke						
Aluminum	<43.15	43.15-80.49	>80.49			
N (cases/controls)	354/347	324/344	356/344			
Model 1[‡]	1.00	0.91 (0.72-1.14)	1.02 (0.81-1.28)	0.72	0.81	1.09 (0.98-1.20)
Model 2[§]	1.00	0.90 (0.71-1.14)	0.99 (0.78-1.26)	0.94	0.94	1.08 (0.97-1.20)
Arsenic	<1.30	1.30-3.03	>3.03			
N (cases/controls)	339/345	320/344	376/346			
Model 1[‡]	1.00	0.97 (0.77-1.22)	1.15 (0.90-1.48)	0.21	0.34	1.02 (0.88-1.18)
Model 2[§]	1.00	0.98 (0.77-1.25)	1.14 (0.88-1.47)	0.29	0.47	1.01 (0.87-1.18)
Barium	<27.40	27.40-56.68	>56.68			
N (cases/controls)	316/345	366/344	353/346			
Model 1[‡]	1.00	1.20 (0.96-1.50)	1.16 (0.91-1.48)	0.27	0.41	1.06 (0.93-1.20)
Model 2[§]	1.00	1.20 (0.95-1.53)	1.09 (0.84-1.41)	0.57	0.73	1.03 (0.90-1.18)
Cobalt	<0.15	0.15-0.19	>0.19			
N (cases/controls)	356/347	326/345	353/343			
Model 1[‡]	1.00	0.91 (0.71-1.16)	0.99 (0.77-1.27)	0.98	0.94	1.01 (0.91-1.11)
Model 2[§]	1.00	0.87 (0.67-1.13)	0.95 (0.73-1.24)	0.77	0.87	0.98 (0.89-1.09)
Copper	<892.63	892.63-1025.91	>1025.91			
N (cases/controls)	299/345	304/345	430/345			
Model 1[‡]	1.00	1.05 (0.83-1.33)	1.56 (1.24-1.97)	<0.001	<0.001	1.30 (1.15-1.46)
Model 2[§]	1.00	1.03 (0.81-1.32)	1.53 (1.20-1.96)	<0.001	<0.001	1.29 (1.13-1.46)

Table III. Adjusted odds ratios (95% CI) for incident stroke according to plasma metals (continued)

Plasma metals	Tertiles of plasma metals (μg/L)			*P*-trend	*FDR*[*]	Linear model[†]
	T1	T2	T3			
Lead	<10.16	10.16-18.62	>18.62			
N (cases/controls)	322/344	338/346	375/344			
Model 1[‡]	1.00	1.08 (0.87-1.34)	1.22 (0.97-1.54)	0.09	0.23	1.12 (1.01-1.24)
Model 2[§]	1.00	1.08 (0.68-1.70)	1.50 (0.92-2.44)	0.09	0.51	1.14 (0.91-1.42)
Manganese	<2.35	2.35-3.43	>3.43			
N (cases/controls)	336/344	334/346	365/345			
Model 1[‡]	1.00	0.98 (0.79-1.23)	1.10 (0.87-1.39)	0.41	0.53	1.05 (0.94-1.18)
Model 2[§]	1.00	1.02 (0.80-1.29)	1.06 (0.83-1.36)	0.62	0.75	1.06 (0.94-1.19)
Mercury	<0.42	0.42-0.71	>0.71			
N (cases/controls)	369/345	351/345	314/345			
Model 1[‡]	1.00	0.93 (0.75-1.16)	0.83 (0.65-1.05)	0.13	0.23	0.97 (0.87-1.09)
Model 2[§]	1.00	0.92 (0.73-1.16)	0.83 (0.64-1.07)	0.16	0.44	0.97 (0.86-1.09)
Molybdenum	<1.09	1.09-1.48	>1.48			
N (cases/controls)	337/345	308/345	389/345			
Model 1[‡]	1.00	0.93 (0.75-1.16)	1.19 (0.95-1.50)	0.10	0.23	1.15 (1.02-1.30)
Model 2[§]	1.00	1.03 (0.82-1.30)	1.24 (0.97-1.57)	0.07	0.43	1.19 (1.05-1.35)
Nickel	<2.04	2.04-3.03	>3.03			
N (cases/controls)	349/347	337/345	349/343			
Model 1[‡]	1.00	0.96 (0.75-1.22)	1.00 (0.78-1.29)	0.93	0.94	1.03 (0.95-1.12)
Model 2[§]	1.00	0.92 (0.72-1.18)	0.98 (0.75-1.28)	0.97	0.94	1.04 (0.95-1.13)
Rubidium	<321.85	321.85-375.09	>375.09			
N (cases/controls)	349/345	351/344	335/346			
Model 1[‡]	1.00	1.00 (0.80-1.24)	0.95 (0.75-1.20)	0.65	0.78	1.04 (0.94-1.15)
Model 2[§]	1.00	0.97 (0.77-1.22)	0.89 (0.70-1.14)	0.35	0.50	1.02 (0.93-1.13)

Table III. Adjusted odds ratios (95% CI) for incident stroke according to plasma metals (continued)

Plasma metals	Tertiles of plasma metals (μg/L)			*P*-trend	*FDR*[*]	Linear model[†]
	T1	T2	T3			
Selenium	<60.75	60.75-74.36	>74.36			
N (cases/controls)	387/344	322/345	326/346			
Model 1[‡]	1.00	0.81 (0.65-1.01)	0.81 (0.64-1.02)	0.07	0.23	0.89 (0.79-1.01)
Model 2[§]	1.00	0.85 (0.67-1.07)	0.87 (0.68-1.11)	0.25	0.47	0.92 (0.82-1.05)
Strontium	<30.25	30.25-38.80	>38.80			
N (cases/controls)	310/344	360/345	365/346			
Model 1[‡]	1.00	1.17 (0.94-1.45)	1.19 (0.96-1.48)	0.13	0.23	1.09 (0.97-1.23)
Model 2[§]	1.00	1.16 (0.93-1.46)	1.12 (0.89-1.41)	0.36	0.50	1.05 (0.93-1.19)
Thallium	<0.08	0.08-0.12	>0.12			
N (cases/controls)	324/344	330/345	381/346			
Model 1[‡]	1.00	1.04 (0.83-1.31)	1.22 (0.96-1.54)	0.09	0.23	1.10 (0.97-1.24)
Model 2[§]	1.00	1.03 (0.81-1.31)	1.17 (0.92-1.49)	0.20	0.44	1.07 (0.95-1.22)
Titanium	<44.48	44.48-53.71	>53.71			
N (cases/controls)	316/345	325/344	394/346			
Model 1[‡]	1.00	1.23 (0.93-1.64)	1.63 (1.18-2.26)	0.003	0.027	1.34 (1.11-1.62)
Model 2[§]	1.00	1.25 (0.93-1.68)	1.60 (1.14-2.25)	0.006	0.05	1.30 (1.07-1.59)
Tungsten	<0.04	0.04-0.06	>0.06			
N (cases/controls)	308/344	393/346	334/345			
Model 1[‡]	1.00	1.40 (1.08-1.80)	1.18 (0.88-1.59)	0.36	0.50	1.02 (0.90-1.17)
Model 2[§]	1.00	1.46 (1.12-1.90)	1.24 (0.90-1.69)	0.26	0.47	1.02 (0.89-1.17)
Vanadium	<1.21	1.21-1.69	>1.69			
N (cases/controls)	317/344	351/346	367/345			
Model 1[‡]	1.00	1.24 (0.94-1.65)	1.37 (1.00-1.88)	0.07	0.23	1.08 (0.93-1.25)
Model 2[§]	1.00	1.31 (0.97-1.77)	1.35 (0.97-1.88)	0.14	0.44	1.06 (0.91-1.24)

Table III. Adjusted odds ratios (95% CI) for incident stroke according to plasma metals (continued)

Plasma metals	Tertiles of plasma metals (μg/L)			*P*-trend	*FDR*[*]	Linear model[†]
	T1	T2	T3			
Zinc	<1120.59	1120.59-2030.87	>2030.87			
N (cases/controls)	338/344	320/344	377/345			
Model 1[‡]	1.00	0.94 (0.74-1.21)	1.14 (0.90-1.44)	0.13	0.23	1.09 (0.97-1.22)
Model 2[§]	1.00	0.89 (0.69-1.15)	1.10 (0.86-1.41)	0.19	0.44	1.06 (0.94-1.20)
Hemorrhagic stroke						
Aluminum	<44.88	44.88-90.31	>90.31			
N (cases/controls)	92/89	104/91	72/89			
Model 1[‡]	1.00	1.08 (0.70-1.68)	0.77 (0.49-1.22)	0.17	0.42	0.83 (0.67-1.02)
Model 2[§]	1.00	1.21 (0.75-1.96)	0.76 (0.47-1.24)	0.16	0.51	0.82 (0.65-1.02)
Arsenic	<1.24	1.24-3.23	>3.23			
N (cases/controls)	89/90	92/90	88/89			
Model 1[‡]	1.00	1.03 (0.66-1.61)	1.02 (0.63-1.65)	0.96	0.94	0.90 (0.66-1.22)
Model 2[§]	1.00	0.97 (0.60-1.57)	0.93 (0.56-1.56)	0.80	0.85	0.86 (0.63-1.20)
Barium	<28.42	28.42-54.12	>54.12			
N (cases/controls)	104/90	73/89	92/90			
Model 1[‡]	1.00	0.71 (0.46-1.10)	0.86 (0.54-1.39)	0.50	0.60	0.98 (0.77-1.26)
Model 2[§]	1.00	0.74 (0.46-1.17)	0.77 (0.46-1.28)	0.28	0.51	0.98 (0.75-1.28)
Cobalt	<0.14	0.14-0.19	>0.19			
N (cases/controls)	80/89	86/90	103/90			
Model 1[‡]	1.00	1.13 (0.74-1.75)	1.41 (0.87-2.28)	0.16	0.42	1.11 (0.91-1.37)
Model 2[§]	1.00	1.09 (0.68-1.73)	1.36 (0.80-2.32)	0.25	0.51	1.09 (0.88-1.36)

Table III. Adjusted odds ratios (95% CI) for incident stroke according to plasma metals (continued)

Plasma metals	Tertiles of plasma metals (μg/L)			*P*-trend	*FDR*[*]	Linear model[†]
	T1	T2	T3			
Copper	<936.36	936.36-1073.46	>1073.46			
N (cases/controls)	89/90	89/89	91/90			
Model 1[‡]	1.00	1.00 (0.65-1.54)	1.04 (0.66-1.64)	0.87	0.92	0.96 (0.76-1.20)
Model 2[§]	1.00	1.13 (0.70-1.81)	1.06 (0.64-1.74)	0.81	0.94	0.96 (0.75-1.23)
Lead	<9.96	9.96-18.24	>18.24			
N (cases/controls)	84/89	80/90	105/90			
Model 1[‡]	1.00	0.94 (0.61-1.45)	1.32 (0.84-2.08)	0.20	0.42	1.09 (0.89-1.34)
Model 2[§]	1.00	1.08 (0.68-1.70)	1.50 (0.92-2.44)	0.09	0.51	1.14 (0.91-1.42)
Manganese	<2.36	2.36-3.60	>3.60			
N (cases/controls)	99/90	94/89	76/90			
Model 1[‡]	1.00	0.92 (0.59-1.43)	0.73 (0.46-1.16)	0.18	0.42	0.85 (0.67-1.07)
Model 2[§]	1.00	0.96 (0.60-1.54)	0.72 (0.44-1.18)	0.18	0.51	0.85 (0.66-1.09)
Mercury	<0.38	0.38-0.74	>0.74			
N (cases/controls)	92/90	104/90	73/89			
Model 1[‡]	1.00	1.05 (0.67-1.63)	0.71 (0.42-1.21)	0.21	0.42	0.77 (0.60-1.01)
Model 2[§]	1.00	1.21 (0.75-1.94)	0.74 (0.42-1.30)	0.29	0.51	0.82 (0.62-1.07)
Molybdenum	<1.11	1.11-1.54	>1.54			
N (cases/controls)	83/89	85/90	100/90			
Model 1[‡]	1.00	1.04 (0.67-1.61)	1.20 (0.78-1.85)	0.41	0.52	1.14 (0.91-1.41)
Model 2[§]	1.00	1.00 (0.62-1.62)	1.17 (0.74-1.87)	0.50	0.60	1.14 (0.90-1.44)
Nickel	<2.01	2.01-2.96	>2.96			
N (cases/controls)	102/89	92/90	75/90			
Model 1[‡]	1.00	0.84 (0.52-1.36)	0.62 (0.37-1.04)	0.07	0.42	0.88 (0.74-1.04)
Model 2[§]	1.00	0.72 (0.43-1.20)	0.72 (0.41-1.27)	0.26	0.51	0.88 (0.74-1.06)

Table III. Adjusted odds ratios (95% CI) for incident stroke according to plasma metals (continued)

Plasma metals	Tertiles of plasma metals (μg/L)			*P*-trend	*FDR*[*]	Linear model[†]
	T1	T2	T3			
Rubidium	<331.22	331.22-381.65	>381.65			
N (cases/controls)	118/90	82/89	69/90			
Model 1[‡]	1.00	0.66 (0.43-1.01)	0.54 (0.34-0.86)	0.008	0.07	0.68 (0.52-0.88)
Model 2[§]	1.00	0.69 (0.44-1.09)	0.51 (0.31-0.85)	0.009	0.14	0.66 (0.50-0.87)
Selenium	<62.31	62.31-75.03	>75.03			
N (cases/controls)	120/89	74/91	74/89			
Model 1[‡]	1.00	0.57 (0.37-0.88)	0.54 (0.34-0.86)	0.006	0.07	0.70 (0.53-0.91)
Model 2[§]	1.00	0.51 (0.32-0.81)	0.57 (0.35-0.95)	0.015	0.14	0.68 (0.51-0.91)
Strontium	<29.94	29.94-38.31	>38.31			
N (cases/controls)	72/89	93/90	104/90			
Model 1[‡]	1.00	1.30 (0.85-2.00)	1.45 (0.94-2.24)	0.10	0.42	1.28 (1.01-1.61)
Model 2[§]	1.00	1.15 (0.72-1.81)	1.28 (0.80-2.04)	0.31	0.51	1.19 (0.92-1.53)
Thallium	<0.09	0.09-0.12	>0.12			
N (cases/controls)	105/90	79/90	85/89			
Model 1[‡]	1.00	0.73 (0.47-1.13)	0.78 (0.49-1.24)	0.28	0.47	0.79 (0.62-1.00)
Model 2[§]	1.00	0.72 (0.45-1.16)	0.84 (0.51-1.39)	0.46	0.60	0.82 (0.63-1.07)
Titanium	<38.04	38.04-51.50	>51.50			
N (cases/controls)	98/90	85/89	86/90			
Model 1[‡]	1.00	0.71 (0.38-1.33)	0.65 (0.30-1.44)	0.32	0.48	0.91 (0.54-1.54)
Model 2[§]	1.00	0.70 (0.35-1.40)	0.69 (0.28-1.70)	0.50	0.60	0.91 (0.50-1.64)
Tungsten	<0.04	0.04-0.06	>0.06			
N (cases/controls)	79/89	94/91	96/89			
Model 1[‡]	1.00	1.22 (0.74-2.01)	1.34 (0.79-2.29)	0.29	0.47	1.03 (0.80-1.31)
Model 2[§]	1.00	1.33 (0.78-2.26)	1.39 (0.78-2.47)	0.28	0.51	1.05 (0.81-1.37)

Table III. Adjusted odds ratios (95% CI) for incident stroke according to plasma metals (continued)

Plasma metals	Tertiles of plasma metals (μg/L)			*P*-trend	*FDR*[*]	Linear model[†]
	T1	T2	T3			
Vanadium	<1.20	1.20-1.78	>1.78			
N (cases/controls)	87/90	97/90	85/89			
Model 1[‡]	1.00	1.21 (0.72-2.02)	0.93 (0.53-1.65)	0.75	0.84	0.92 (0.70-1.22)
Model 2[§]	1.00	1.10 (0.64-1.92)	0.94 (0.51-1.71)	0.80	0.85	0.90 (0.67-1.22)
Zinc	<1152.07	1152.07-2135.07	>2135.07			
N (cases/controls)	107/90	79/90	83/89			
Model 1[‡]	1.00	0.65 (0.40-1.06)	0.75 (0.47-1.19)	0.39	0.52	0.95 (0.78-1.15)
Model 2[§]	1.00	0.77 (0.45-1.29)	0.78 (0.48-1.27)	0.43	0.60	0.94 (0.76-1.16)

Abbreviations: FDR-False Discovery Rate.

[*]False Discovery Rate (FDR) adjusted *p*-values were calculated using software published by Pike (2011).

[†]Linear model: Odds ratios (95% CI) for incident stroke corresponding to an interquartile range increase in plasma metal levels were shown.

[‡]Model 1: Metals were included in the conditional logistic regression models separately without adjustment.

[§]Model 2: Metals were included in the conditional logistic regression models separately and adjusted for body mass index (kg/m^2), smoking status (current, former, never), drinking status (current, former, never), regular exercise, family history of stroke, hyperlipidemia, diabetes and hypertension

Table IV. Adjusted odds ratios (95% CI) for incident stroke according to plasma metals in sensitivity analysis

Plasma metals	Tertiles of plasma metals (μg/L)			*P*-trend	Linear model*
	T1	T2	T3		
Ischemic stroke					
Copper	<892.21	892.21-1025.81	>1025.81		
N (cases/controls)	261/298	263/297	366/297		
Model 1†	1.00	1.03 (0.80-1.33)	1.52 (1.18-1.95)	0.001	1.29 (1.13-1.46)
Model 2‡	1.00	1.00 (0.77-1.30)	1.47 (1.13-1.91)	0.002	1.26 (1.10-1.44)
Molybdenum	<1.08	1.08-1.48	>1.48		
N (cases/controls)	287/298	272/297	332/297		
Model 1†	1.00	0.96 (0.76-1.22)	1.20 (0.94-1.53)	0.13	1.17 (1.03-1.33)
Model 2‡	1.00	1.05 (0.81-1.35)	1.23 (0.95-1.59)	0.11	1.18 (1.03-1.35)
Selenium	<60.46	60.46-74.00	>74.00		
N (cases/controls)	332/297	272/297	288/298		
Model 1†	1.00	0.80 (0.63-1.01)	0.83 (0.65-1.07)	0.15	0.91 (0.80-1.04)
Model 2‡	1.00	0.86 (0.67-1.11)	0.89 (0.68-1.15)	0.37	0.94 (0.83-1.08)
Titanium	<44.32	44.32-53.63	>53.63		
N (cases/controls)	272/298	284/296	336/298		
Model 1†	1.00	1.28 (0.94-1.75)	1.66 (1.17-2.37)	0.004	1.34 (1.09-1.64)
Model 2‡	1.00	1.30 (0.94-1.79)	1.63 (1.12-2.37)	0.010	1.28 (1.04-1.59)
Hemorrhagic stroke					
Rubidium	<334.13	334.13-383.40	>383.40		
N (cases/controls)	103/77	66/77	61/76		
Model 1†	1.00	0.61 (0.39-0.97)	0.56 (0.35-0.92)	0.017	0.64 (0.48-0.85)
Model 2‡	1.00	0.67 (0.41-1.09)	0.55 (0.32-0.95)	0.027	0.62 (0.46-0.85)
Selenium	<62.60	62.60-75.03	>75.03		
N (cases/controls)	108/76	60/78	61/76		
Model 1†	1.00	0.50 (0.31-0.81)	0.48 (0.29-0.80)	0.003	0.68 (0.51-0.91)
Model 2‡	1.00	0.47 (0.28-0.79)	0.54 (0.31-0.93)	0.013	0.69 (0.50-0.94)

Metals which were significantly associated with incident stroke (*P-trend* <0.05) or selected by elastic net model were included in the table.

*Linear model: Odds ratios (95% CI) for incident stroke corresponding to an interquartile range increase in plasma metal levels were shown.

†Model 1: Metals were included in the conditional logistic regression models separately without adjustment.

‡Model 2: Metals were included in the conditional logistic regression models separately and adjusted for body mass index (kg/m^2), smoking status (current, former, never), drinking status (current, former, never), regular exercise, family history of stroke, hyperlipidemia, diabetes and hypertension.

Table V. Adjusted odds ratios (95% CI) for subtypes of stroke according to plasma metals

Plasma metals	Tertiles of plasma metals (μg/L)			*P* for trend	Linear model*
	T1	T2	T3		
Large artery atherosclerosis infarction (n=382)					
Copper	1 [Reference]	1.16 (0.77, 1.75)	2.01 (1.32, 3.06)	0.001	1.36 (1.09, 1.70)
Molybdenum	1 [Reference]	1.21 (0.81, 1.80)	1.11 (0.74, 1.66)	0.66	1.12 (0.91, 1.38)
Selenium	1 [Reference]	1.04 (0.70, 1.55)	1.15 (0.77, 1.74)	0.49	1.01 (0.80, 1.29)
Titanium	1 [Reference]	1.11 (0.68, 1.82)	1.28 (0.73, 2.24)	0.39	1.24 (0.87, 1.76)
Cardioembolic infarction (n=206)					
Copper	1 [Reference]	0.86 (0.47, 1.59)	1.08 (0.57, 2.05)	0.75	1.17 (0.87, 1.58)
Molybdenum	1 [Reference]	1.29 (0.72, 2.30)	1.52 (0.84, 2.75)	0.18	1.28 (0.95, 1.73)
Selenium	1 [Reference]	0.97 (0.56, 1.65)	0.67 (0.37, 1.23)	0.21	0.87 (0.62, 1.23)
Titanium	1 [Reference]	0.94 (0.45, 1.95)	1.50 (0.65, 3.45)	0.30	1.29 (0.77, 2.16)
Lacunar infarction (n=228)					
Copper	1 [Reference]	0.63 (0.37, 1.06)	0.61 (0.35, 1.05)	0.07	1.33 (1.00, 1.77)
Molybdenum	1 [Reference]	0.61 (0.37, 1.03)	1.06 (0.64, 1.75)	0.72	1.15 (0.85, 1.56)
Selenium	1 [Reference]	0.78 (0.48, 1.28)	0.88 (0.51, 1.50)	0.60	1.00 (0.83, 1.20)
Titanium	1 [Reference]	2.91 (1.21, 6.97)	4.97 (1.83, 13.53)	0.002	1.42 (0.94, 2.12)
Undetermined cause of infarction (n=219)					
Copper	1 [Reference]	0.97 (0.56, 1.66)	1.36 (0.81, 2.31)	0.22	1.33 (1.00, 1.77)
Molybdenum	1 [Reference]	1.29 (0.74, 2.24)	2.00 (1.09, 3.65)	0.024	1.38 (1.03, 1.84)
Selenium	1 [Reference]	0.71 (0.40, 1.24)	0.78 (0.45, 1.36)	0.40	0.78 (0.58, 1.06)
Titanium	1 [Reference]	0.69 (0.33, 1.43)	1.20 (0.52, 2.76)	0.63	1.32 (0.83, 2.11)
Intracerebral hemorrhage (n=233)					
Rubidium	1 [Reference]	0.61 (0.37, 1.01)	0.55 (0.33, 0.93)	0.020	0.69 (0.51, 0.92)
Selenium	1 [Reference]	0.57 (0.35, 0.92)	0.51 (0.30, 0.88)	0.010	0.68 (0.50, 0.92)
Subarachnoid hemorrhage (n=36)					
Rubidium	1 [Reference]	1.87 (0.32, 10.81)	0.61 (0.05, 7.58)	0.98	0.44 (0.10, 1.90)
Selenium	1 [Reference]	1.02 (0.26, 4.03)	0.03 (0.00, 1.57)	0.09	0.47 (0.12, 1.84)

Metals associated with incident stroke (*P*-trend<0.05) or selected by elastic net model were included.

Metals were included in the conditional logistic regression models separately and adjusted for body mass index (kg/m^2), smoking status (current, former, never), drinking status (current, former, never), physical activity, family history of stroke, hyperlipoidemia, diabetes and hypertension.

*Linear model: Odds ratios (95% CI) for incident stroke corresponding to an interquartile range increase in plasma metal levels were shown.

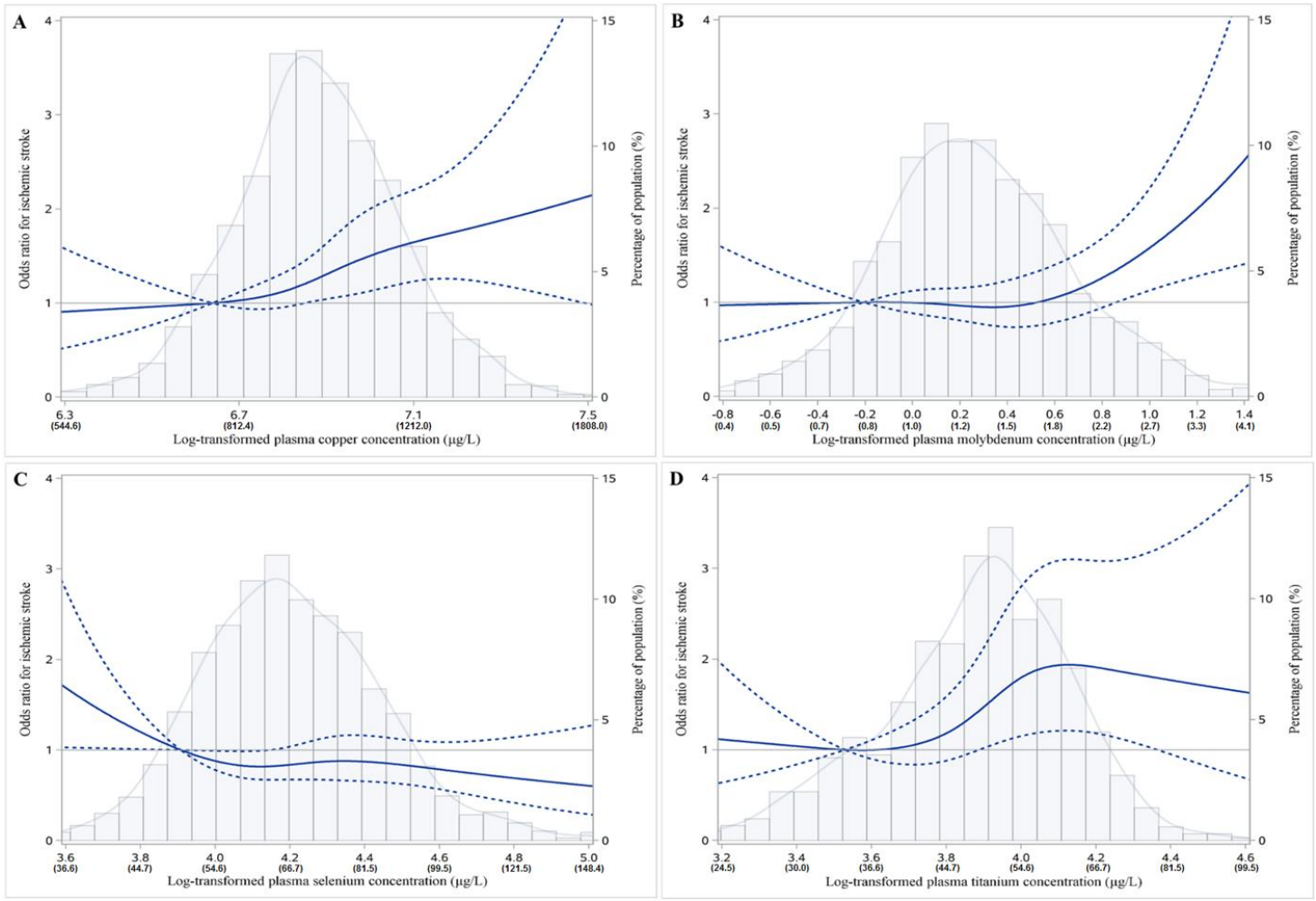

Figure I. The restricted cubic spline for the associations of plasma metals with incident ischemic stroke in sensitivity analysis

The restricted cubic spline for the associations of plasma copper (A) molybdenum (B) selenium (C) titanium (D) with ischemic stroke. The bars represent histograms of plasma metal distribution among the total population. The lines represent adjusted odds ratios for the log transformed levels of plasma metals in the conditional regression model. Knots were placed at the 20th 40th 60th and 80th percentiles of the plasma metal distribution and the reference value was set at the percentile of 10th. Models were adjusted for body mass index (kg/m^2), smoking status (current, former, never), drinking status (current, former, never), regular exercise, family history of stroke, hyperlipidemia, diabetes and hypertension.

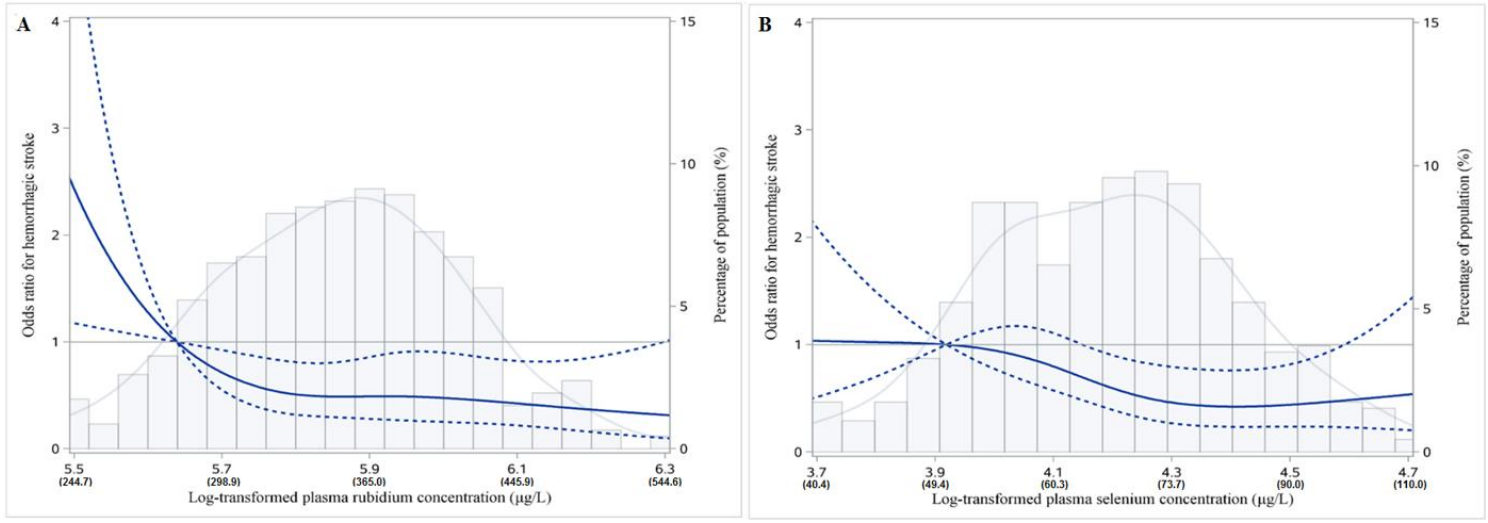

Figure II. The restricted cubic spline for the associations of plasma metals with incident hemorrhagic stroke in sensitivity analysis

The restricted cubic spline for the associations of plasma rubidium (A), selenium (B) with hemorrhagic stroke. The bars represent histograms of plasma metal distribution among the total population. The lines represent adjusted odds ratios for the log transformed levels of plasma metals in the conditional regression model. Knots were placed at the 20th 40th 60th and 80th percentiles of the plasma metal distribution and the reference value was set at the percentile of 10th. Models were adjusted for body mass index (kg/m^2), smoking status (current, former, never), drinking status (current, former, never), regular exercise, family history of stroke, hyperlipidemia, diabetes and hypertension.

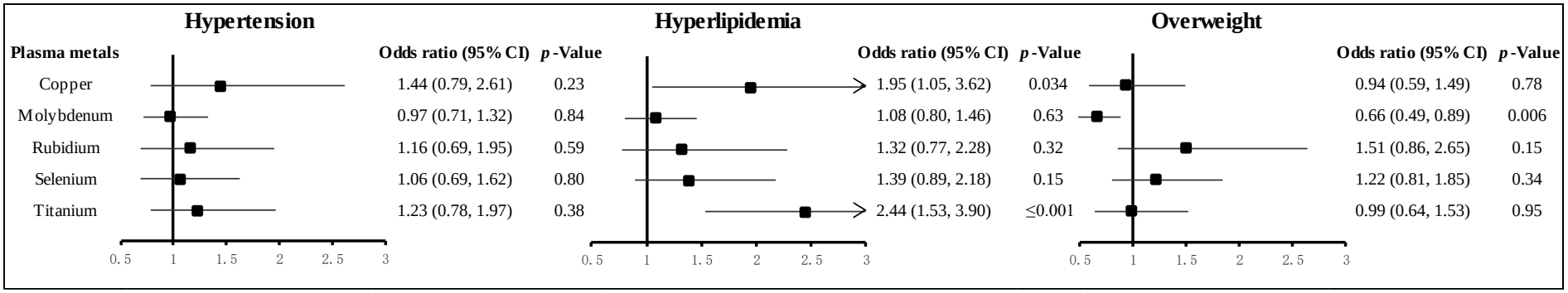

Figure III. Adjusted odds ratios (95% CI) for risk of hypertension, hyperlipidemia and overweight according to plasma metals

Natural log-transformed values of metal concentrations were included in the unconditional logistic regression models separately and adjusted for age, sex, smoking status (current, former, never), drinking status (current, former, never), regular exercise. Body mass index (kg/m^2) and family history of hypertension were additionally adjusted for hypertension. Body mass index (kg/m^2) and family history of hyperlipidemia were additionally adjusted for hyperlipidemia.

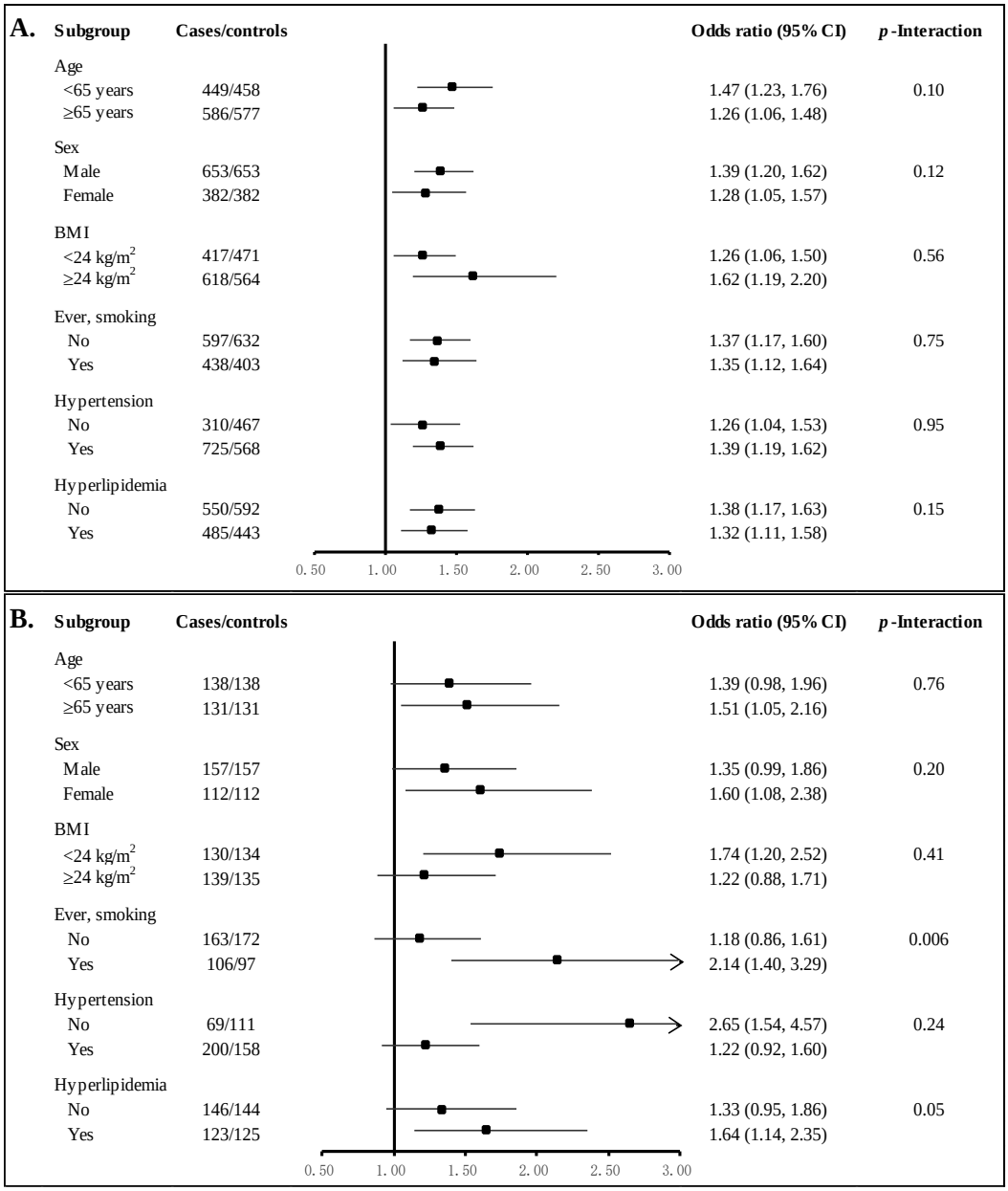

Figure IV. Adjusted odds ratios (95% CI) for incident stroke according to predictive plasma metal scores in subgroups

Adjusted odds ratio for incident ischemic stroke (A) and hemorrhagic stroke (B) according to predictive plasma metal scores were analyzed in subgroup stratified by age, sex, body mass index, ever-smokers (included current and former smokers), hypertension and hyperlipidemia. Unconditional logistic regression models were used and all stratified models were adjusted for age, sex, body mass index (kg/m^2), smoking status (current, former, never), drinking status (current, former, never), regular exercise, family history of stroke, hyperlipidemia, diabetes and hypertension.

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2. Yuan Y, Xiao Y, Feng W, Liu Y, Yu Y, Zhou L, et al. Plasma metal concentrations and incident coronary heart disease in Chinese adults: the Dongfeng-Tongji cohort. *Environ Health Perspect*. 2017;125:107007.