SUPPLEMENTARY MATERIALS

Supplementary Methods

Study Population

This study used baseline data from the Guangxi Eco-Environmental Health and Aging Study (GEHAS), an ongoing population-based cohort established in 2018 among rural residents in Guangxi, China. Participants aged ≥ 30 years who completed the baseline survey during 2018–2019 were eligible for inclusion. All participants were recruited from villages in the study area according to a unified field protocol. Therefore, the study population is more representative of adults living in similar rural areas of Guangxi than of the general Chinese population. A total of 4,356 individuals were initially enrolled.

Participants were excluded if they had substantial missing questionnaire data, missing physical examination or biochemical data, or insufficient plasma metal measurements. We also excluded individuals with self-reported physician-diagnosed chronic hepatitis, cirrhosis, liver cancer, or other major liver disease, major kidney disease, thyroid disorders, cancer, or medications affecting liver function. Liver function biomarkers were not included in the exclusion criteria or outcome definition in the present analysis. After these exclusions, 3,116 participants remained in the final analysis. The participant selection process is shown in Supplementary Figure S1.

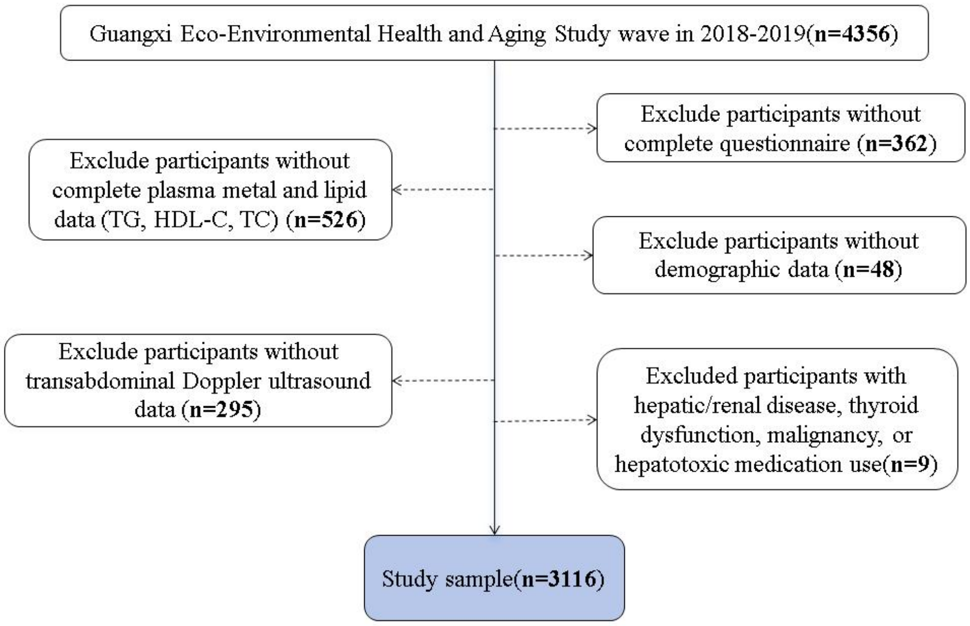

Figure S1. Participant selection and exclusion details

Plasma Metal Assessments

Plasma concentrations of the 10 metals were measured by inductively coupled plasma mass spectrometry (ICP-MS; Thermo Fisher Scientific, ICAPRQ 01408, USA) according to previously described procedures[1]. Detailed sample pretreatment procedures, including nitric acid dilution and instrument cleaning, are provided in Supplementary Table S1. The intra- and inter-assay coefficients of variation for plasma metal measurements were both less than 10%. Standard addition recovery rates ranged from 80.2% to 114.7% across all samples, and the results for the quality control material are shown in Supplementary Table S2. The limits of detection (LOD) ranged from 0.002 to 0.071 μg/L, and values below the LOD were imputed as LOD/√2[2].

Supplementary Table S1. Protocol for metal concentration measurements

Parameters	Conditions
Analysis time	3 times
Uptake time	30 s
Plasma exhaust	0.3–0.7 mbar
Penning pressure	< 5×10–7 mbar
STD autotune sensitivity	Li > 50,000, Co > 50,000, In > 240,000, U > 330,000, CeO/Ce < 3%
KED autotune sensitivity	Co > 30,000, Co/ClO > 18
Elements	Ca^{44}, Fe^{57}, Co^{59}, Cu^{63}, Zn^{66}, As^{75}, Mo^{95}, Cd^{111}, Ba^{138}, Pb^{208}

Supplementary Table S2 Certification documentation for metal concentration analysis

Element	Level	Certified Value (μg/L)	Recovery (%)	Acceptable Range (μg/L)

Fe (mg/L)	Level I	0.885	88.13	0.752–1.020
	Level II	1.240		1.050–1.420
Co	Level I	2.040	82.47	1.630– 2.440
	Level II	9.130		7.300– 11.000
Cu	Level I	0.733	81.44	0.623– 0.843
	Level II	1.260		1.080–1.450
Zn(mg/L)	Level I	1.760	91.98	1.500–2.020
	Level II	2.130		1.810– 2.450
As	Level I	10.600	89.22	8.470–12.700
	Level II	48.100		38.500– 57.800
Cd	Level I	2.270	85.55	1.820–2.730
	Level II	10.700		8.570–12.900
Ba	Level I	727.000	92.61	618.000–836.000
	Level II	794.000		675.000–913.000
Mo	Level I	1.910	86.73	1.340–2.480
	Level II	6.580		5.270–7.900

Assessment of MASLD

MASLD was defined according to diagnostic criteria established for the Chinese population [3]. Participants were classified as having MASLD if hepatic steatosis was confirmed by imaging and at least one metabolic risk criterion was present. These criteria included body mass index (BMI) ≥ 23 kg/m² or waist circumference > 90 cm in men or > 80 cm in women[4]; fasting plasma glucose (FPG) ≥ 5.6 mmol/L, 2-hour postprandial glucose (P2BG) ≥ 7.8 mmol/L, glycated hemoglobin (HbA1c) ≥ 5.7%, a diagnosis type 2 diabetes mellitus, or use of antidiabetic medication; blood pressure ≥ 130/85 mmHg or use of antihypertensive medication; triglyceride (TG) levels ≥ 1.7 mmol/L or use of lipid-lowering medication; or high-density lipoprotein cholesterol (HDL-C) ≤ 1.0 mmol/L in male or 1.3 mmol/L in female, or use of lipid-lowering medication. Fibrosis assessment was not included in the primary diagnostic definition used in the present analysis.

Assessment of Lipid Biomarkers

All participants fasted for at least 12 hours before the physical examination, and fasting venous blood samples were collected on the same day. Serum biochemical indicators were measured using a Hitachi 7600-020 automated biochemical analyzer (Hitachi, Tokyo, Japan). TG concentrations were measured directly, and the TC/HDL-C ratio was calculated as total cholesterol divided by HDL-C. These two indicators were selected a priori as representative lipid biomarkers of metabolic dysfunction because of their well-established relevance to hepatic steatosis and cardiometabolic risk [5–8].

Covariates Definition

Information on covariates was collected through face-to-face interviews by trained staff using a standardized baseline questionnaire, and the covariates were selected on the basis of relevant literature [9]. Participants reported their age and sex, and ethnicity was categorized as Han, Yao, Zhuang, or other. Educational attainment was classified as below primary school, primary school, and middle school or above. Marital status was categorized as unmarried, married, or widowed. Smoking status was defined as consuming at least 1 cigarette per day for ≥ 6 months, and alcohol consumption as drinking alcoholic beverages at least once per week during the previous 6 months. Both behaviors were coded as binary variables (yes or no). Obesity was defined as BMI ≥ 28 kg/m^2.

Statistical Analysis

Baseline characteristics were summarized according to variable type. Continuous variables with a normal distribution are presented as the mean ± standard deviation (SD), whereas non-normally distributed variables are presented as the median and interquartile range (IQR). Categorical variables are presented as frequencies (percentages). Because several plasma metal concentrations were right-skewed, all metal concentrations were natural log-transformed [Ln(X+1)] before analysis. Between-group differences were performed using the Kruskal-Wallis H test for continuous variables or the chi-square (χ^2) test for categorical variables.

Multivariable logistic regression models were used to examine the linear associations of individual metal exposure, TG, and the TC/HDL-C ratio with MASLD. Multivariable linear regression models were used to evaluate the associations of individual metal concentrations with TG and the TC/HDL-C ratio. TG and TC/HDL-C were categorized into quartiles only when their associations with MASLD were evaluated. Two models were fitted for each analysis: Model 1 was unadjusted, and Model 2 was adjusted for sex, age, ethnicity, educational attainment, smoking status, alcohol drinking, obesity, and diabetes. Restricted cubic spline (RCS) regression with four knots was used to explore potential nonlinear associations between plasma metal concentrations and MASLD. For metals showing evidence of nonlinearity, piecewise linear regression models were further used to identify potential threshold effects, and model fit was evaluated using likelihood ratio tests. Given the exploratory nature of this study, Bonferroni correction for multiple comparisons was not applied.

Exploratory mediation analysis was conducted to assess whether TG and TC/HDL-C might statistically account for part of the associations between metal exposures and MASLD. Both the exposure-mediator and exposure-outcome models were adjusted for the covariates included in Model 2. Indirect and direct effect estimates were summarized by the ACME and ADE generated under the mediation analysis framework, and the proportion explained was calculated [10]. Because the exposure, lipid biomarkers, and MASLD were measured at the same baseline examination, these analyses were interpreted as hypothesis-generating rather than causal.

All analyses were conducted using R software (version 4.5.0), with RCS, threshold, and mediation analyses were conducted using the “rms”, “segmented”, and “mediation” packages, respectively. A two-sided $P < 0.05$ was considered statistically significant.

Supplementary Table S3. Basic characteristics of the study population (N = 3,116)

	All (*N* = 3,116)	**No-MASLD (*n* = 2,662)**	**MASLD (*n* = 454)**	***P* value**
Age (%)				< 0.001
< 60 years	1,655 (53.1)	1,375 (51.7)	280 (61.7)	
≥ 60 years	1,461 (46.9)	1,287 (48.3)	174 (38.3)	
Sex (%)				< 0.001
Female	1,940 (62.3)	1,730 (65.0)	210 (46.3)	
Male	1,176 (37.7)	932 (35.0)	244 (53.7)	
Ethnicity (%)				0.022
Han	636 (20.4)	563 (21.1)	73 (16.1)	
Yao	2,337 (75.0)	1,973 (74.1)	364 (80.2)	
Zhuang and others	143 (4.6)	126 (4.7)	17 (3.7)	
Education (%)				< 0.001
Below primary school level	1,255 (40.3)	1,112 (41.7)	143 (31.5)	
Primary school graduation	751 (24.1)	643 (24.2)	108 (23.8)	
Middle school and above	1,110 (35.6)	907 (34.1)	203 (44.7)	
Smoking (%)				0.031
No	2,538 (81.5)	2,185 (82.1%)	353 (77.8)	
Yes	578 (18.5)	477 (17.9%)	101 (22.2)	
Drink (%)				< 0.001
No	2,111 (67.7)	1,838 (69.0)	273 (60.1)	
Yes	1,005 (32.3)	824 (31.0%)	181 (39.9)	
Diabetes (%)				< 0.001
No	2,988 (95.9)	2574 (96.7)	414 (91.2)	
Yes	128 (4.1)	88 (3.3)	40 (8.8)	
Obesity (%)				<0.001
No	2,909 (93.4)	2571 (96.6)	338 (74.4)	
Yes	207 (6.6)	91 (3.4)	116 (25.6)	
TG (mmol/L)	1.05 [0.74, 1.60]	0.98 [0.71, 1.43]	1.77 [1.14,2.56]	< 0.001
TC/HDL-C	3.16 [2.70, 3.73]	3.08 [2.65, 3.61]	3.73 [3.27, 4.21]	< 0.001

Supplementary Table S4. Distribution of metal concentrations

	All (*N* = 3116)	**Non-MASLD (*n* = 2,662)**	**MASLD (*n* = 454)**	***P* value**
Ca (mg/L)	70.59 [66.72, 75.04]	70.41 [66.63, 74.68]	71.74 [67.43, 76.74]	< 0.001
Co (μg/L)	0.25 [0.16, 0.48]	0.26 [0.16, 0.51]	0.23 [0.15, 0.38]	0.002
Cu (mg/L)	0.92 [0.80, 1.04]	0.92 [0.81, 1.05]	0.89 [0.79, 1.01]	0.001
Zn (mg/L)	1.06 [0.76, 4.34]	1.04 [0.76, 3.97]	1.21 [0.82, 5.85]	< 0.001
As (μg/L)	1.19 [0.92, 1.88]	1.19 [0.92, 1.88]	1.18 [0.92, 1.96]	0.896
Mo (μg/L)	1.59 [1.22, 2.07]	1.60 [1.23, 2.08]	1.51 [1.15, 2.03]	0.010
Cd (μg/L)	0.19 [0.13, 0.27]	0.19 [0.13, 0.27]	0.19 [0.13, 0.28]	0.962
Ba (μg/L)	24.10 [17.23, 33.02]	23.75 [17.15, 32.57]	26.10 [17.88, 36.69]	0.002
Pb (μg/L)	5.14 [3.30, 8.61]	5.14 [3.30, 8.55]	5.12 [3.34, 8.85]	0.675
Fe (mg/L)	1.08 [0.83, 1.36]	1.06 [0.81, 1.34]	1.18 [0.93, 1.47]	< 0.001

Supplementary Table S5. Multivariable logistic regression results examining associations of ten metals with MASLD

	Group	Model 1		Model 2	
		OR (95% *CI*)	*P* value	*OR* (95% *CI*)	*P* value
Cd	*Q2*	0.96 (0.72–1.27)	0.773	0.91 (0.67–1.24)	0.552
	Q3	1.01 (0.76–1.34)	0.943	1.03 (0.76–1.39)	0.857
	Q4	1.01 (0.76–1.34)	0.943	1.03 (0.76–1.39)	0.869
	Continuous	0.80 (0.35–1.76)	0.595	1.02(0.42–2.34)	0.973
Pb	*Q2*	1.05 (0.79–1.40)	0.720	0.92 (0.68–1.24)	0.580
	Q3	0.95 (0.71–1.26)	0.714	0.85 (0.62–1.15)	0.293
	Q4	1.11 (0.84–1.46)	0.477	0.90 (0.67–1.22)	0.507
	Continuous	1.02(0.87–1.18)	0.814	0.94(0.79–1.12)	0.513
Ca	*Q2*	1.00 (0.74–1.36)	1.000	0.99 (0.71–1.37)	0.950
	Q3	1.31 (0.98–1.76)	0.066	1.30 (0.95–1.79)	0.096
	Q4	1.69 (1.28–2.25)	< 0.001	1.68 (1.25–2.29)	< 0.001
	Continuous	1.83(0.96–3.72)	0.083	1.57(0.80–3.35)	0.224
Ba	*Q2*	0.97 (0.72–1.30)	0.821	0.92 (0.67–1.26)	0.605
	Q3	1.14 (0.85–1.52)	0.379	1.08 (0.79–1.47)	0.633
	Q4	1.44 (1.09–1.91)	0.009	1.22 (0.90–1.67)	0.207
	Continuous	1.39(1.11–1.74)	0.004	1.21(0.95–1.55)	0.131
Fe	*Q2*	1.40 (1.03–1.91)	0.035	1.48 (1.06–2.07)	0.022
	Q3	1.53 (1.13–2.09)	0.006	1.52 (1.10–2.13)	0.013
	Q4	2.18 (1.64–2.93)	< 0.001	1.85 (1.34–2.56)	< 0.001
	Continuous	1.87(1.45–2.42)	< 0.001	1.55(1.18–2.05)	0.002
Mo	*Q2*	0.72 (0.55–0.95)	0.022	0.77 (0.57–1.03)	0.082
	Q3	0.76 (0.57–0.99)	0.045	0.86 (0.64–1.16)	0.328
	Q4	0.70 (0.53–0.92)	0.012	0.82 (0.61–1.12)	0.214
	Continuous	0.98(0.79–1.17)	0.802	0.96(0.78–1.16)	0.690
Co	*Q2*	1.22 (0.94–1.60)	0.138	1.11 (0.83–1.49)	0.469
	Q3	0.77 (0.58–1.03)	0.080	0.72 (0.52–0.98)	0.040
	Q4	0.74 (0.55–0.98)	0.040	0.74 (0.54–1.01)	0.058
	Continuous	0.32(0.17–0.61)	< 0.001	0.32(0.16–0.63)	0.001
Cu	*Q2*	0.95 (0.73–1.25)	0.733	1.09 (0.81–1.46)	0.557
	Q3	0.80 (0.61–1.06)	0.123	0.98 (0.72–1.33)	0.903
	Q4	0.63 (0.47–0.84)	0.002	0.79 (0.57–1.10)	0.163
	Continuous	0.59(0.41–0.82)	0.002	0.71(0.50–1.01)	0.046
Zn	*Q2*	1.44 (1.07–1.94)	0.017	1.39 (1.01–1.92)	0.045
	Q3	1.30 (0.96–1.76)	0.092	1.32 (0.95–1.82)	0.096
	Q4	1.80 (1.35–2.40)	< 0.001	1.71 (1.25–2.36)	< 0.001
	Continuous	1.16(1.06–1.27)	< 0.001	1.14 (1.03–1.26)	0.008
As	*Q2*	0.94 (0.71–1.25)	0.667	0.94 (0.69–1.27)	0.692
	Q3	0.92 (0.69–1.22)	0.565	0.88 (0.65–1.19)	0.404
	Q4	1.00 (0.76–1.32)	1.000	0.92 (0.68–1.24)	0.576
	Continuous	1.02(0.81–1.28)	0.841	0.94(0.72–1.20)	0.610

Supplementary Table S6. Results from segmented linear regression analysis demonstrating breakpoint effects of heavy metal exposure on MASLD

Metals	Group	Segment	*OR* (95%*CI*)	*P* value	Breakpoint	AIC	Chisq	*Pr (> Chisq)*
Ba	Low	Ba ≤ breakpoint	0.748 (0.425–1.341)	0.320	3.163 ± 0.189	7.481	4.851	0.088
	High	Ba > breakpoint	1.692 (0.956–2.975)	0.069				
Mo	Low	Mo ≤ breakpoint	0.067 (0.006–0.768)	0.027	0.742 ± 0.049	2.996	12.883	0.001
	High	Mo > breakpoint	1.063 (0.867–1.279)	0.533				

Note. Breakpoints indicate concentration inflection points (μg/L) from segmented linear regression. AIC values represent model fit. Likelihood ratio tests (*Chi-square*, *P*-values) compare segmented and linear models; $P < 0.05$ indicates significant threshold effects. All models were adjusted for sex, age, ethnicity, educational attainment, smoking status, alcohol consumption, obesity, and diabetes.

Supplementary Table S7. Multivariable linear regression results examining associations of ten metals with two lipid metabolism indicators

Group		Model 1		Model 2	
		B (95% *CI*)	*P* value	*B* (95% *CI*)	*P* value
TG	Ca	0.09 (0.02–0.15)	0.011	0.08 (0.02–0.15)	0.015
	Mo	0.00 (-0.02–0.02)	0.898	0.00 (-0.02–0.02)	0.938
	As	0.04 (0.01–0.07)	0.003	0.03 (0.01–0.06)	0.015
	Ba	0.00 (-0.03–0.03)	0.994	0.00 (-0.03–0.02)	0.855
	Pb	-0.01 (-0.03–0.01)	0.319	-0.01 (-0.03–0.01)	0.322
	Co	-0.11 (-0.18–-0.04)	0.003	-0.11 (-0.18–-0.04)	0.002
	Cu	0.01 (-0.04–0.05)	0.715	0.01 (-0.03–0.06)	0.623
	Zn	0.01 (0.00–0.02)	0.308	0.01 (0.00–0.02)	0.250
	Fe	0.03 (0.00–0.05)	0.038	0.03 (0.00–0.05)	0.039
	Cd	-0.06 (-0.15–0.04)	0.225	-0.04 (-0.14–0.05)	0.366
TC/HDL–C	Ca	0.21 (0.06–0.35)	0.005	0.18 (0.04–0.33)	0.011
	Mo	0.01 (-0.04–0.06)	0.662	0.00 (-0.05–0.05)	0.977
	As	0.13 (0.07–0.19)	< 0.001	0.10 (0.04–0.16)	0.001
	Ba	0.03 (-0.03–0.09)	0.338	0.00 (-0.05–0.06)	0.880
	Pb	-0.01 (-0.05–0.03)	0.471	-0.02 (-0.06–0.02)	0.264
	Co	-0.27 (-0.43–-0.11)	0.001	-0.28 (-0.43–-0.12)	< 0.001
	Cu	0.12 (0.02–0.22)	0.019	0.19 (0.09–0.29)	< 0.001
	Zn	0.01 (-0.01–0.04)	0.276	0.01 (-0.01–0.04)	0.383
	Fe	0.04 (-0.01–0.10)	0.118	0.03 (-0.03–0.08)	0.369
	Cd	-0.43 (-0.64–-0.23)	< 0.001	-0.40 (-0.60–-0.20)	< 0.001

Supplementary Table S8. Multivariable logistic regression results examining associations of two lipid metabolism parameters with MASLD

Group		Model 1		Model 2	
		OR (95% *CI*)	*P* value	*OR* (95% *CI*)	*P* value
TC/HDL-C	*Q*2	1.17 (0.77–1.78)	0.458	1.07 (0.70–1.64)	0.756
	*Q*3	3.32 (2.34–4.79)	< 0.001	2.96 (2.06–4.32)	< 0.001
	*Q*4	7.00 (5.03–9.95)	< 0.001	5.38 (3.80–7.77)	< 0.001
TG	*Q*2	2.28 (1.49–3.55)	< 0.001	2.29 (1.48–3.61)	< 0.001
	*Q*3	3.52 (2.36–5.39)	< 0.001	3.36 (2.22–5.21)	< 0.001
	*Q*4	11.47 (7.93–17.13)	< 0.001	10.35 (7.04–15.68)	< 0.001

Supplementary Table S9. Decomposition of mediation effects

Mediator	Metal	ADE	*P* value	ACME	*P* value	Prop. Mediated	*P* values	Prop. Mediated (%)
TG	Co	-0.09 (-0.13–-0.02)	0.014	-0.02 (-0.04–-0.01)	0.004	0.26 (0.10–0.55)	0.012	26.20
TG	Fe	0.004 (0.00–0.01)	< 0.001	0.001 (0.00004–0.003)	0.038	0.99 (0.007–0.33)	0.038	16.70
TC/HDL-C	Co	-0.07 (-0.12–-0.02)	0.004	–0.027 (-0.043–0.012)	< 0.001	0.26 (0.11–0.53)	< 0.001	25.40

Note. Models were adjusted for sex, age, ethnicity, educational attainment, smoking status, alcohol consumption, obesity, and diabetes. ACME represents the Average Causal Mediation Effect, and ADE represents the Average Direct Effect. Because the data were cross-sectional, these results should be interpreted as exploratory and hypothesis-generating rather than causal. TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol.

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