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Association between serum Copper-Zinc-Selenium mixture and multiple health outcomes

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ABSTRACT

Background: Metallic biomaterials have transformed modern medicine, with copper (Cu), zinc (Zn), and selenium (Se) emerging as critical components in medical applications. The study of the single and synergistic effects of serum metal concentrations on human health can provide valuable insights for future clinical transformation of biodegradable alloys.

Methods: We evaluated 2381 NHANES 2011–2016 participants to study individual and combined effects of these metals on health outcomes. Multivariable logistic regression, restricted cubic splines, and piecewise linear regression were used to examine linear, nonlinear, and threshold relationships. Overall metal mixture effects were assessed using weighted quantile sum (WQS) and Bayesian kernel-machine regression (BKMR).

Results: Elevated serum Cu levels were significantly associated with an increased risk of osteoarthritis. When Serum Cu $\geq$ 99.48 μg/dL, each 1-unit increase in Ln Cu raised diabetes risk 4.55-fold. For Se $\geq$ 122.74 μg/L, each 1-unit increase in Ln Se led to a 29.96-fold rise in diabetes prevalence, for Se $<$ 157.56 μg/L it increased heart attack risk 165.19-fold. Furthermore, mixtures of Cu, Se, and Zn were positively associated with diabetes, hypertension, and heart attack risks; each unit increase in the mixture corresponded to a 23 % rise in diabetes and a 15 % rise in hypertension prevalence.

Conclusions: Serum Cu levels $\geq$99.48 μg/dL are significantly linked to diabetes risk, while serum Se levels $\geq$122.74 μg/L are associated with diabetes risk and levels $<$157.56 μg/L with elevated heart attack risk. Serum metal mixtures containing Cu, Se and Zn were significantly and positively associated with risk of diabetes, hypertension and heart attack.

1. Introduction

With advances in biomedical engineering and material science, the integration of metallic biomaterials into modern medicine has dramatically changed human contact with metals. Approximately 70–80 % of all medical implants are made of metallic biomaterials, underscoring their critical role in healthcare [1]. Among various metallic elements, copper (Cu), zinc (Zn), and selenium (Se) have emerged as particularly significant components in newly invented medical applications, especially through applications, such as orthopedic implants [2,3], cardiovascular devices [4], and therapeutic nanoparticles [5–7]. Although medical metallic materials are widely utilized owing to their excellent properties, the degradation of these metallic materials releases ions, which may have adverse effects on human health, including suppression of immune responses and increased risks of chronic diseases [1,8]. Previous studies have found serum metal exposure to be associated with

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diabetes, neurodegenerative diseases, cardiovascular disease, arthritis, and even cancer [9–12].

However, current serum metal studies have predominantly employed single-metal exposure paradigms, which fail to reflect the clinical reality that patients are frequently exposed to multiple metallic components through various medical devices and implants throughout their lifetime. Various metal-based alloys have been extensively studied and are considered promising biodegradable materials. Zn-Cu alloys have shown excellent biodegradability and antibacterial properties in vitro and in animal model experiments, making them suitable for medical devices, such as gynecological catheters, cardiovascular stents, and orthopedic implants [13,14]. Although Se is not commonly used in traditional metal alloys, studies have shown that nanoselenium or Se-doped coatings can enhance antioxidant and anti-infective properties [15], suggesting that selenium can be used in biodegradable zinc-copper alloys. In the future, shape memory alloys are expected to have a wide range of potential medical applications [16]. Simultaneous exposure to multiple metals may result in complex synergistic or antagonistic health effects [17] that conventional single-metal studies cannot capture. A paucity of systematic investigations examining the cumulative impact of Cu-Zn-Se mixtures on multiple health outcomes is present. This knowledge gap significantly limits our understanding of the potential health risks associated with exposure to multiple metals in clinical settings.

Building on previous studies on serum Zn concentrations and health outcomes [18], we expanded our investigation by integrating emerging epidemiological evidence into the evolving field of medical metal-based materials. In this comprehensive analysis, we examined the associations between serum Cu, Se, and Zn mixtures and chronic health outcomes using nationally representative data from the National Health and Nutrition Examination Survey (NHANES) 2011–2016. The selected health outcomes (diabetes, cardiovascular disease, cancer, and arthritis) were chosen to align with the principal clinical applications of metallic biomaterials. To better reflect clinical realities, we implemented a set of complementary analytical methods to assess both the individual metal effects and overall interactions within mixtures. We investigated the associations between single serum metal concentrations and health outcomes using multivariate logistic regression and restricted cubic spline regression for nonlinear associations. We then performed threshold effect tests to identify possible concentration thresholds for individual metals. For the overall analysis of mixtures, we estimated their effects on health outcomes using weighted quantile sum regression and Bayesian kernel regression. The robustness of our findings was verified through single-metal studies using Bayesian kernel regression and quantile-based g calculations for the directional heterogeneity of mixture effects.

2. Materials and methods

2.1. Study design and population

We analyzed data from the 2011–2016 National Health and Nutrition Examination Survey (NHANES), a nationally representative cross-sectional survey of the United States (U.S.) civilian noninstitutionalized population [19]. The survey included administering questionnaires, conducting physical examinations and household interviews (covering demographic, dietary, and health-related questions and examinations), and performing laboratory tests. Previously published reports have thoroughly described the planning, operation, and design of these surveys [20–23]. To increase statistical power, participant data from three NHANES cycles were combined for multicycle analysis, with weights adjusted accordingly [19]. Of the 29,902 participants, we excluded those aged <20 years (n = 12,854) and those with missing disease (n = 1365), serum metal (n = 10,937), or covariate data (n = 2365). The final analytical sample included 2381 participants for the Cu-Se-Zn mixture analysis (Fig. 1).

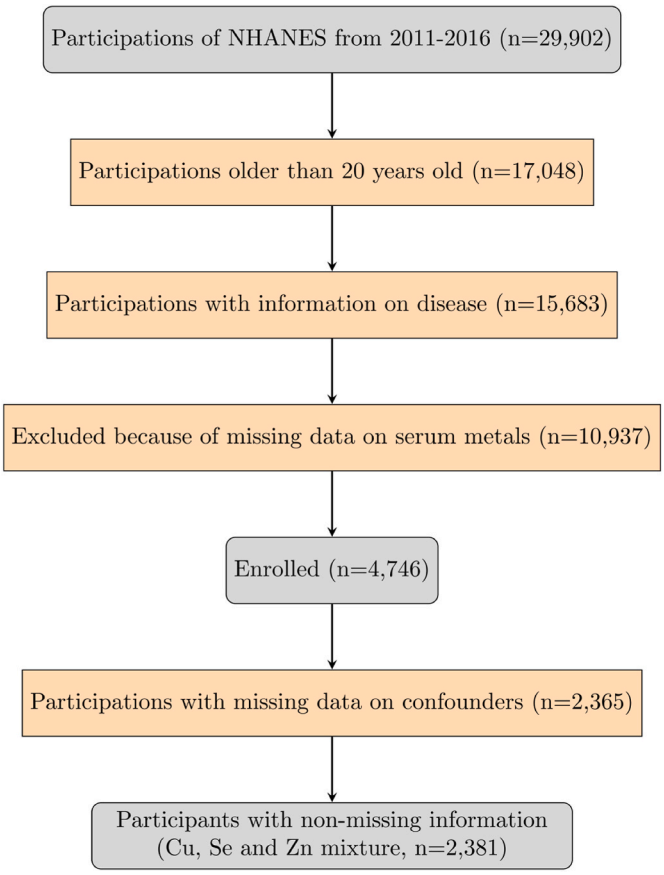

Fig. 1. Flowchart of participant selection in the study.

2.2. Disease assessment

Disease status was determined using a standardized questionnaire. Participants were classified as having a condition if they answered "yes" to the question "Has a doctor or other health professional ever told you that you have [condition]?" These conditions included diabetes mellitus, coronary heart disease (CHD), hypertension, heart attack, cancer, osteoarthritis, and rheumatoid arthritis (RA). Early osteoarthritis was defined as a diagnosis before 55 years of age.

2.3. Metal exposure assessment

Serum levels of three metals (Cu, Zn, and Se) were measured from 2011 to 2016, with detection rates exceeding 75 %. Values below the limit of detection were imputed as LOD/$\sqrt{2}$ (detection rates and LODs are provided in Supplementary Table S1). Detailed instructions on specimen collection and processing are available on the NHANES website.[19]

2.4. Covariates

We adjusted for demographic characteristics (age [20–29, 30–39, 40–49, 50–59 years], sex, race/ethnicity [Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, non-Hispanic Asian, other]), body mass index (<18.5, 18.5–25, 25–30, 30–35, 35–40, >40 kg/m^2), socioeconomic factors (education level [less than high school, high school graduate/some college, college graduate or above], poverty-income ratio [≤1, 1–3, >3]), and lifestyle factors (smoking status [every day, some days, not at all, unknown], physical activity [<500, 500–1000, ≥1000 MET-min/week], and sedentary time [<4, 4–6, 6–8, ≥8 h/day]). All information was collected during household interviews

[19].

2.5. Statistical analysis

We calculated survey-weighted proportions for baseline characteristics and presented metal concentrations as medians with interquartile ranges. Metal concentrations were natural log-transformed due to their right-skewed distributions.

Considering the complex NHANES survey design, weighted proportions were calculated to compare the baseline characteristics across different metal mixture exposure groups. Serum metal concentrations were divided into quartiles, and chi-square tests were used for categorical variables. Due to the skewed distribution of metals, we applied a natural logarithmic transformation.

The analysis was conducted in three phases. In the first phase, we focused on the association between serum metal levels and health outcomes. Multiple logistic regression analyses were performed using log-transformed serum metal concentrations as continuous variables. Three models were used: Model 1 (unadjusted), Model 2 (adjusted for age, sex, and race/ethnicity), and Model 3 (fully adjusted for age, sex, race/ethnicity, education level, poverty-to-income ratio, body mass index, smoking status, physical activity, and sedentary time). Odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated for the effects of each serum metal on health outcome and presented as forest plots.

To investigate nonlinear associations between serum metal concentrations and health outcomes, we used a restricted cubic spline (RCS) function to analyze OR relationships [24]. Additionally, to assess the potential threshold effects, we implemented segmented linear regression with a smoothing function. The threshold values (turning points) were identified using an iterative algorithm that evaluated predetermined intervals, selected the point maximizing model likelihood, and estimated the effect sizes (ORs) for intervals above and below the identified thresholds.

In the second phase, to investigate the mixed effects of serum metals on each health outcome and their potential impact, we used two complementary methods to assess mixed exposure effects. The weighted quantile sum (WQS) regression method randomly split the data into training and validation sets in a 4:6 ratio, with effect weights for each metal determined through 100 bootstrap iterations [25]. Considering the potential nonlinear effects of plasma metal exposure on the risk of each health outcome and possible interactions between these metals, we applied Bayesian kernel-machine regression (BKMR) with 10,000 iterations to assess the complex exposure-response relationships. The combined effect of mixed serum metal exposure on health outcomes was assessed by comparing the effects of a 5 %-point increase or decrease in exposure relative to the median level [26].

For sensitivity analysis, we performed a single-exposure association analysis using the BKMR in the association analysis between single metals and the risk of health outcomes. Other metals were fixed at the 25th, 50th, and 75th percentiles to estimate their effects. In addition, univariate exposure-response relationships were assessed using BKMR, with other metals were simultaneously fixed at the 50th percentile to estimate individual metal effects. Bivariate exposure-response curves were plotted to visualize plasma metal interactions.[26] Additionally, to assess the mixture effects of plasma metals, addressing the limitations of WQS regression, a quantile-based g-calculation (qgcomp) was used to validate the findings without assuming the same direction of effect between each metal exposure and the outcome. This allowed for the testing of both positive and negative effects in the mixtures [27]. Considering the relatively small sample sizes of patients with CHD and heart attack and based on recommendations from similar previous studies, we combined the CHD, hypertension, and heart attack groups into a cardiovascular disease (CVD) group. We then conducted the same statistical analyses as part of the sensitivity analysis [21].

All analyses accounted for the complex design of the NHANES using appropriate sampling weights and were conducted using R software (version 4.4.1; The R Foundation for Statistical Computing), utilizing the survey package for weighted analyses, gWQS for WQS regression, bkmr for BKMR analysis, rms for RCS regression, and qgcomp for quantile-based g-computation. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Baseline characteristics of participants

A total of 2381 participants from NHANES 2011–2016 were included in this study, including participants with diabetes (n = 174), CHD (n = 17), hypertension (n = 507), heart attack (n = 27), cancer (n = 81), osteoarthritis (n = 114), RA (n = 60) and early-onset osteoarthritis (n = 93). Significant differences were observed across the metal quartiles. Regarding serum copper levels, sex distribution, BMI categories, and smoking status differed significantly (all $p < 0.001$). Regarding serum zinc levels, notable differences were found in the age distribution ($p = 0.105$), race/ethnicity distribution ($p < 0.001$), BMI categories ($p = 0.050$), and smoking status ($p = 0.103$). For serum Se, significant variations were detected in the race/ethnicity distribution ($p = 0.011$) and PIR categories ($p = 0.143$). All analyses used weighted data to account for the complex design of NHANES (Table 1).

3.2. Associations between single serum metal exposure and health outcomes

Univariate and multivariate regression analyses were performed to investigate the association between natural log-transformed metal concentrations and multiple health outcomes. Notably, in the fully adjusted model (Model III), each unit increase in serum Cu levels was linked to 42 % higher odds of osteoarthritis (OR = 1.42, 95 % CI: 1.08–1.87). Similarly, each unit increase in serum Zn levels was associated with 15 % lower odds of hypertension (OR = 0.85, 95 % CI: 0.73–0.98) and every unit increase in serum Se levels was associated with 28 % lower odds of diabetes (OR = 0.72, 95 % CI: 0.58–0.89). Table 2 presents associations between all other serum metals and multiple health outcomes.

For the threshold effect of serum metal exposure levels on health outcomes, the RCS plot (Fig. 2) showed nonlinear relationships between serum metal exposure levels and the risk of multiple health outcomes. Significantly, the OR (95 % CI) for diabetes prevalence (Table 3) was 4.550 (0.147–141.099) for each 1 unit increase in Ln Cu after the turning point (Ln Cu = 4.60, Cu = 99.48 μg/dL). Similarly, the OR (95 % CI) for diabetes prevalence (Table 3) was 29.961 ($0.085–1.06 \times 10^4$) for each 1 unit increase in Ln Se after the turning point (Ln Se = 4.81, Se = 122.74 μg/L). However, the OR (95 % CI) for heart attack prevalence (Table 3) was 165.193 ($2.520–1.08 \times 10^4$) for each 1 unit increase in LnSe before the turning point (Ln Se = 5.06, Se = 157.56 μg/L). In other cases, some of the results did not reach statistical significance but suggested potential clinical relevance (p-values close to 0.05). The threshold effect results are presented in Table 3.

3.3. Mixed effects of serum metals on multiple health outcomes

WQS regression revealed differential contributions of each serum metal to the mixture across health outcomes (Fig. 3). Cu showed the highest positive weights for diabetes (weigh t = 0.689), hypertension (weight = 0.705), heart attack (weight = 0.732), and RA (weight = 0.832), whereas Zn demonstrated protective effects against cancer (weight = 0.737). The joint effect of serum metal mixture on diabetes showed that each unit increase was significantly associated with 23 % higher odds (OR = 1.23, 95 % CI: 1.09–1.38, $p < 0.001$). Also, every unit increase in the serum metal mixture was significantly associated with 15 % higher hypertension (OR = 1.15, 95 % CI: 1.07–1.24, $p < 0.001$). Other weights for health outcomes are shown in Table S2 of the

Table 1
Baseline characteristics of the study population according to metal mixtures (weighted).

Characteristics	Serum Copper				P	Serum Zinc				P	Serum Selenium				P
	Q1	Q2	Q3	Q4		Q1	Q2	Q3	Q4		Q1	Q2	Q3	Q4	
Sex					<0.001					<0.001					<0.001
Male	83.8	60.9	45.9	12.0		50.5	48.2	46.9	42.2		42.2	46.9	48.2	59.3	
Female	16.2	39.1	54.1	88.0		49.5	51.8	53.1	57.8		57.8	53.1	51.8	40.7	
Age					0.040					0.105					0.066
20–29	31.8	25.9	23.0	29.7		25.7	33.1	36.5	36.5		35.2	32.8	31.2	31.9	
30–39	25.2	25.2	23.2	25.9		32.4	35.9	31.2	31.5		34.2	32.9	39.5	32.4	
40–49	23.6	24.8	23.3	23.6		22.4	24.0	18.6	20.5		21.7	19.9	21.3	22.0	
50–59	19.4	24.0	30.5	20.8		19.5	7.0	13.7	11.5		8.9	14.4	8.0	13.7	
Race					<0.001					<0.001					0.011
Mexican American	8.2	11.2	10.6	10.6		8.2	11.2	10.6	10.6		11.2	10.4	9.1	9.9	
Other Hispanic	6.5	5.9	7.0	7.8		6.5	5.9	7.0	7.8		7.4	7.4	6.8	5.5	
Non-Hispanic White	66.9	66.6	64.4	57.7		66.9	66.6	64.4	57.7		57.9	62.1	68.8	66.9	
Non-Hispanic Black	5.5	8.0	11.7	17.5		5.5	8.0	11.7	17.5		12.9	10.5	8.2	10.3	
Non-Hispanic Asian	9.4	5.7	3.9	4.0		9.4	5.7	3.9	4.0		7.2	5.8	4.6	6.0	
Other Race	3.4	2.6	2.5	2.4		3.4	2.6	2.5	2.4		3.4	3.7	2.6	1.4	
BMI					<0.001					0.050					0.047
<18.5	2.1	1.8	1.5	1.2		1.8	1.6	1.4	1.9		1.7	1.5	1.6	1.8	
18.5–24.9	35.2	32.8	31.2	31.9		33.1	32.4	31.5	31.7		32.8	31.9	32.4	33.5	
25.0–29.9	34.2	32.9	39.5	32.4		35.9	34.2	33.8	34.9		34.9	35.2	34.8	34.2	
30.0–39.9	21.7	19.9	21.3	22.0		20.5	22.4	21.7	20.8		21.3	22.0	21.5	20.9	
≥40	6.8	12.6	6.5	12.5		8.7	9.4	11.6	10.7		9.3	9.4	9.7	9.6	
Education					0.390					0.170					0.436
Less than high school	10.6	12.2	14.7	11.3		9.1	14.2	11.5	13.9		12.3	12.8	10.9	12.7	
High school graduate or some college	51.6	53.3	54.2	53.3		51.7	52.8	53.4	54.3		53.5	54.3	49.6	54.9	
College graduate or above	37.7	34.5	31.1	35.4		39.2	33.1	35.1	31.8		34.2	32.9	39.5	32.4	
PIR					0.001					0.686					0.143
<1	11.4	12.4	19.5	20.3		16.2	17.3	15.0	14.4		16.2	17.3	15.0	14.4	
1–3	36.7	33.3	34.6	34.2		34.2	39.0	31.0	34.9		34.2	39.0	31.0	34.9	
≥3	52.0	54.3	45.9	45.6		49.7	43.7	53.9	50.7		49.7	43.7	53.9	50.7	
Smoking					<0.001					0.104					0.336
Every day	9.6	16.3	21.9	15.3		14.1	14.8	15.5	18.4		20.2	12.3	13.7	16.6	
Some days	6.2	3.9	6.9	4.3		4.4	6.2	4.5	6.2		5.6	5.6	5.3	5.0	
Not at all	18.3	19.4	19.8	12.9		13.1	20.1	18.7	18.8		16.3	18.1	17.8	18.6	
Unknown	65.9	60.3	51.4	67.5		68.5	58.9	61.3	56.7		57.9	64.0	63.2	59.8	
Physical activity					0.001					0.722					0.870
MET<500	25.7	33.1	36.5	36.5		32.4	35.9	31.2	31.5		35.2	32.8	31.2	31.9	
MET 500–1000	9.0	11.6	8.4	13.5		12.6	9.2	9.0	11.8		10.5	11.3	11.8	8.8	
MET ≥1000	65.3	55.3	55.1	50.0		55.1	54.9	59.8	56.8		54.3	55.9	57.0	59.3	
Sedentary time					0.155					0.413					0.362
<4h	33.3	34.1	31.2	33.4		28.6	33.6	35.9	33.4		31.1	34.5	35.9	30.7	
4–6h	19.3	22.6	24.1	19.0		22.4	24.0	18.6	20.5		21.7	19.9	21.3	22.0	
6–8h	18.4	18.9	19.0	19.9		17.1	17.4	21.7	19.5		17.6	20.9	17.0	20.4	
>8h	29.1	24.3	25.7	27.8		31.9	25.1	23.9	26.6		29.6	24.8	25.7	26.8	
Comorbidities															
Diabetes	5.3	4.6	8.2	7.5	0.155	6.8	5.9	6.4	6.5	0.103	5.5	4.1	5.4	9.9	0.005
CHD	1.5	0.6	0.9	1.0	0.736	3.8	3.6	2.7	3.2	0.647	3.8	3.6	2.7	3.2	0.647
Hypertension	18.9	19.1	23.7	22.0	0.736	18.3	18.3	22.2	24.2	0.797	20.0	15.3	22.7	24.6	0.362
Heart attack	0.1	1.1	0.8	2.0	0.736	1.2	0.9	1.0	0.9	0.886	1.1	0.8	1.2	0.9	0.886
Cancer	5.1	2.7	7.4	2.0	0.004	5.7	4.6	3.1	4.1	0.719	6.1	3.6	6.3	6.4	0.012
Osteoarthritis	2.9	4.8	4.6	5.6	0.004	4.8	4.2	4.5	4.4	0.559	4.6	4.3	4.1	4.9	0.559
Rheumatoid arthritis	1.9	2.3	1.4	3.4	0.736	4.3	4.2	3.9	4.5	0.930	4.3	4.2	3.9	4.5	0.930
early-onset Osteoarthritis	2.9	4.8	4.6	5.6	0.371	3.8	4.9	3.5	5.5	0.647	5.2	2.7	4.6	5.1	0.647

Values are presented as weighted percentages.
P values were calculated using weighted chi-square tests.
Abbreviations: BMI, body mass index; PIR, poverty income ratio; MET, metabolic equivalent; CHD, coronary heart disease.

Supplementary Materials.

Using the BKMR model, we assessed the mixed effects of serum metal mixtures on each health outcome. The combined effects of mixed serum metal exposure on health outcomes are shown in Table 4. For diabetes, hypertension, and heart attack, metal mixtures containing Cu, Se, and Zn were significantly associated with increased disease risk. Despite negative trends in metal mixture effects on cancer and RA, these associations were not statistically significant. The metal mixtures exhibited varying effects on arthritic conditions, with suggestive positive associations for osteoarthritis and early osteoarthritis. The BKMR results for each health outcome is detailed in Fig. S1 of the supplementary materials.

3.4. Sensitivity analyses

BKMR also showed similar results for the effect of a single serum metal on health outcome risk (Table 4). Serum Cu levels had a significant positive effect on the prevalence of hypertension. Serum Zn levels showed a significant positive effect on osteoarthritis risk when Cu and Se levels were above the 50th percentile. In diabetes, serum Se levels showed a significant positive effect when Cu and Zn levels were above the 50th percentile. However, serum Se levels showed a significant negative effect on cancer risk when Cu and Zn levels were below the 50th percentile. Details are shown in Fig. S1 of the Supplementary Materials.

Quantile G-computation analysis confirmed the relative importance

Table 2
Association between single heavy metal and multiple health outcomes, ORs, NHANES, 2011–2018.

Subgroups	Model 1 (95% CI)	Model 2 (95% CI)	Model 3 (95% CI)
DM			
Cu	1.47 (0.50 − 4.36)	1.67 (0.30 − 9.20)	0.72 (0.17 − 3.04)
Se	9.22 (1.72 − 49.35)	8.30 (1.43 − 8.06)	9.45 (1.50 − 59.55)
Zn	1.20 (0.50 − 2.86)	1.22 (0.45 − 3.31)	1.14 (0.38 − 3.39)
CHD			
Cu	0.48 (0.07 − 3.44)	1.13 (0.14 − 9.35)	0.08 (0.00 − 1.23)
Se	17.24 (0.71 − 419.65)	14.31 (0.38 − 541.32)	3.21 (0.03 − 326.35)
Zn	3.06 (0.67 − 13.93)	2.94 (0.47 − 18.49)	2.01 (0.22 − 18.13)
HBP			
Cu	1.18 (0.70 − 1.98)	2.05 (1.01 − 4.16)	1.32 (0.67 − 2.61)
Se	3.74 (1.45 − 9.67)	3.05 (1.08 − 8.63)	2.97 (0.97 − 9.15)
Zn	1.64 (0.81 − 3.30)	1.43 (0.66 − 3.11)	1.34 (0.57 − 3.13)
HA			
Cu	5.30 (2.08 − 13.51)	14.51 (3.01 − 70.01)	5.89 (0.33 − 105.98)
Se	14.99 (1.96 − 114.87)	13.31 (1.99 − 89.12)	8.95 (0.91 − 88.47)
Zn	4.98 (0.57 − 43.63)	5.43 (0.50 − 58.92)	8.61 (0.55 − 135.66)
Ca			
Cu	0.82 (0.30 − 2.23)	0.54 (0.13 − 2.25)	0.49 (0.10 − 2.37)
Se	0.03 (0.00 − 0.28)	0.02 (0.00 − 0.26)	0.02 (0.00 − 0.19)
Zn	0.45 (0.11 − 1.79)	0.32 (0.06 − 1.61)	0.27 (0.05 − 1.57)
OA			
Cu	0.93 (0.29 − 2.93)	0.48 (0.12 − 1.85)	0.33 (0.10 − 1.06)
Se	0.75 (0.11 − 5.27)	0.59 (0.10 − 3.60)	0.82 (0.09 − 7.36)
Zn	1.67 (0.51 − 5.51)	1.79 (0.41 − 7.78)	1.78 (0.38 − 8.36)
RA			
Cu	1.79 (0.50 − 6.35)	2.07 (0.41 − 10.58)	1.30 (0.18 − 9.15)
Se	3.69 (0.12 − 117.23)	2.81 (0.09 − 6.20)	2.31 (0.18 − 30.41)
Zn	0.52 (0.12 − 2.27)	0.44 (0.08 − 2.34)	0.57 (0.10 − 3.26)
eOA			
Cu	1.05 (0.25 − 4.40)	0.53 (0.10 − 2.90)	0.30 (0.08 − 1.23)
Se	0.52 (0.05 − 5.80)	0.46 (0.05 − 4.34)	0.71 (0.05 − 10.37)
Zn	2.06 (0.53 − 7.98)	2.52 (0.50 − 12.62)	2.53 (0.48 − 13.50)

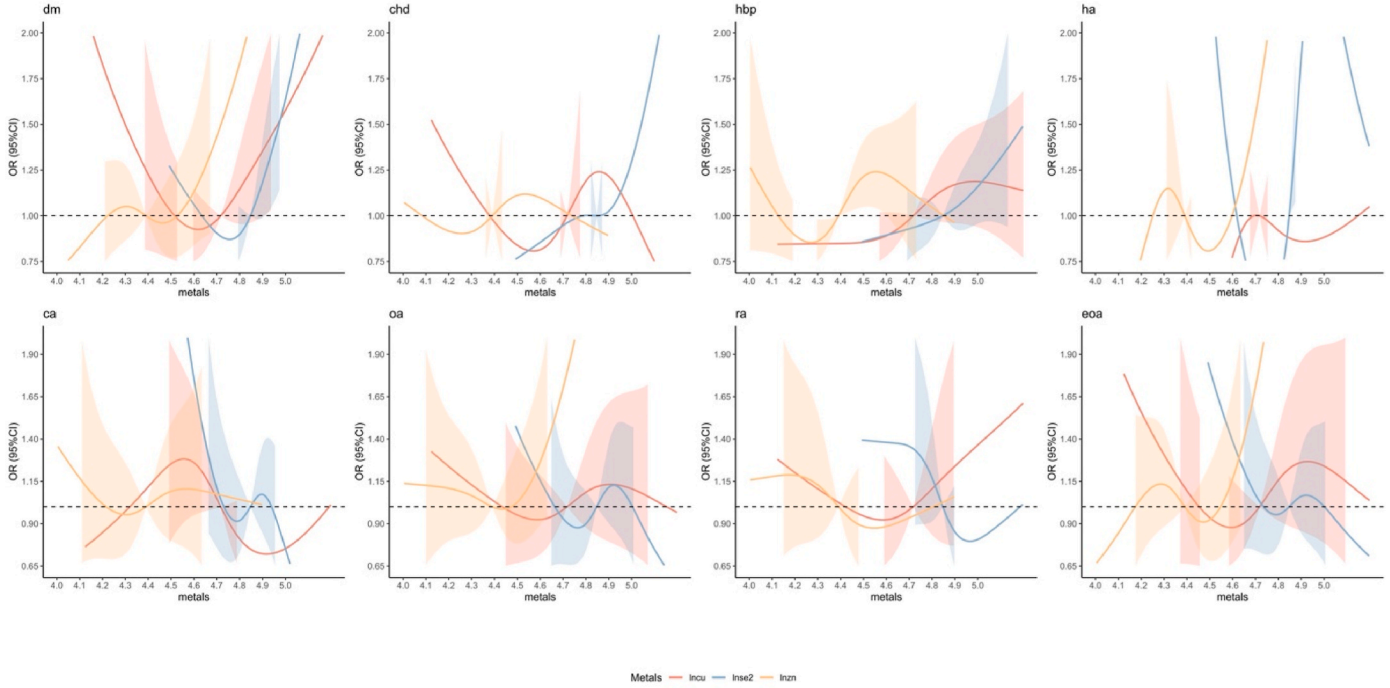

Fig. 2. Associations between each serum metal concentration (Ln transformed) with health outcomes evaluated by RCS.
This model adjusted for all covariates. Cu, copper; Zn, zinc; Se, selenium; DM, diabetes mellitus; CHD, coronary heart disease; HBP, hypertension; HA, heart attack; Ca, cancer; OA, osteoarthritis; RA, rheumatoid arthritis; eOA, early-onset osteoarthritis.

Table 3
Threshold effect analysis of serum metals on health outcomes using segmented linear regression.

Serum metal	OR	95 % CI	P-value
Diabetes			
Ln Cu < 4.60	0.146	0.015–1.443	0.100
Ln Cu > 4.60	4.550	0.147–141.099	0.008[a]
Ln Se < 4.81	0.387	0.008–19.706	0.636
Ln Se > 4.81	29.961	0.085–1.06 × 10^4	0.050[a]
Ln Zn < 4.53	1.397	0.341–5.728	0.642
Ln Zn > 4.53	7.638	0.154–377.805	0.360
Coronary heart disease			
Ln Cu < 5.14	1.692	0.073–39.188	0.743
Ln Cu > 5.14	0.000	0.000–Inf	0.866
Ln Se < 4.92	1.390	0.001–1.84 × 10^3	0.928
Ln Se > 4.92	44.104	0.000–8.59 × 10^6	0.491
Ln Zn < 4.10	Inf	0.000–Inf	0.900
Ln Zn > 4.10	0.672	0.000–Inf	0.900
Hypertension			
Ln Cu < 4.39	0.365	0.010–13.415	0.583
Ln Cu > 4.39	1.735	0.010–297.639	0.405
Ln Se < 4.79	1.086	0.064–18.377	0.955
Ln Se > 4.79	2.930	0.044–194.497	0.530
Ln Zn < 4.19	0.091	0.003–2.381	0.150
Ln Zn > 4.19	2.142	0.020–233.263	0.065
Heart attack			
Ln Cu < 4.60	5.36 × 10^8	0.000–1.29 × 10^{36}	0.266
Ln Cu > 4.60	0.791	0.000–4.75 × 10^{21}	0.262
Ln Se < 5.06	165.193	2.520–1.08 × 10^4	0.017[a]
Ln Se > 5.06	0.000	0.000–5.18 × 10^{308}	0.523
Ln Zn < 4.54	1.738	0.043–70.045	0.770
Ln Zn > 4.54	44.149	0.024–7.98 × 10^4	0.331
Cancer			
Ln Cu < 5.15	0.353	0.096–1.299	0.117
Ln Cu > 5.15	150.283	0.274–8.24 × 10^4	0.055
Ln Se < 4.98	0.251	0.027–2.355	0.226
Ln Se > 4.98	0.000	0.000–982.803	0.391
Ln Zn < 4.69	1.448	0.326–6.438	0.627
Ln Zn > 4.69	0.000	0.000–1.35 × 10^{12}	0.462
Osteoarthritis			
Ln Cu < 4.39	0.079	0.001–6.582	0.261
Ln Cu > 4.39	1.435	0.002–833.084	0.214
Ln Se < 4.64	0.008	0.000–5.75 × 10^3	0.482
Ln Se > 4.64	1.106	0.000–2.34 × 10^8	0.477
Ln Zn < 4.51	0.705	0.122–4.070	0.696
Ln Zn > 4.51	21.934	0.432–1.11 × 10^3	0.055
Rheumatoid arthritis			
Ln Cu < 5.15	3.997	0.699–22.855	0.119
Ln Cu > 5.15	0.000	0.000–9.68 × 10^7	0.458
Ln Se < 5.06	0.213	0.021–2.136	0.189
Ln Se > 5.06	10.623	0.000–2.53 × 10^5	0.435
Ln Zn < 4.69	0.845	0.162–4.406	0.841
Ln Zn > 4.69	0.000	0.000–6.58 × 10^{17}	0.629
Early - onset arthritis			
Ln Cu < 4.39	0.044	0.001–3.276	0.156
Ln Cu > 4.39	1.492	0.003–739.589	0.124
Ln Se < 4.64	0.002	0.000–1.70 × 10^3	0.368
Ln Se > 4.64	0.931	0.000–2.96 × 10^8	0.378
Ln Zn < 4.48	0.833	0.097–7.165	0.868
Ln Zn > 4.48	24.626	0.387–1.57 × 10^3	0.062

This model was adjusted for all covariates. Cu, copper; Zn, zinc; Se, selenium. Inf, infinity.
[a] P-value<0.05.

of individual metals in both directions. Cu had the highest positive weight for cardiovascular outcomes, whereas Se had the highest negative weight for metabolic disorders. The details are shown in Fig. S2 of the Supplementary Materials.

Considering the relatively small sample sizes of patients with CHD (n = 17) and heart attack (n = 27), we conducted a sensitivity analysis by combining CHD, heart attack, and hypertension (n = 507) into a CVD group. In the multivariate logistic regression models, Se was identified as a significant risk factor for CVD prevalence across all three adjusted models (Model III, OR = 4.00, 95 % CI: 1.34–12.00). The results of the RCS and segmented linear regression models showed no significant threshold points for serum metal content in relation to CVD prevalence. In the BKMR analysis of metal mixtures, the serum Cu-Se-Zn mixture demonstrated an overall positive effect on CVD prevalence, with the ratios between each pair of these three metals influencing disease occurrence. Additional details are provided in Tables S3 and S4 and Figs. S3–S6 of the Supplementary Materials.

4. Discussion

In this study, we systematically investigated the synergistic effects and complex regulatory mechanisms of Cu, Zn, and Se on human health. The study findings revealed the multifaceted physiological functions and potential interactions between these trace elements in disease progression and development.

Our results indicate that participants with serum Cu levels ≥99.48 μg/dL have a significantly increased risk of developing diabetes. Cu, a crucial metalloenzyme cofactor, plays a critical role in various biological processes, including cellular redox regulation, by modulating antioxidant enzyme activity and metallothionein expression, thereby affecting cellular oxidative stress levels. Copper homeostasis is crucial for human health, as both copper deficiency and excess can have adverse effects. Its metabolic dysregulation is closely associated with diabetes, cardiovascular diseases, and cancer [28–30].

A previous cross-sectional study involving 3472 Chinese participants found that Cu was significantly positively correlated with HbA1c ($\beta = 0.324$, $p < 0.05$) and was more significant in women, individuals over 60 years, and non-smokers [31]. Another prospective study following 1911 Finnish men found that elevated serum Cu levels were significantly associated with an increased risk of CVD-related death, with a more pronounced effect in individuals with obesity [32]. Disorders in Cu homeostasis are also associated with multiple cancer types, likely due to the important role of Cu-binding protein kinases in tumor occurrence, progression, and metastasis [33]. In addition, Cu homeostasis imbalance can promote the development of atherosclerosis through multiple mechanisms, such as oxidative stress, inflammation, and endothelial dysfunction [34]. Cu can be used as part of Zn-Cu degradable alloy materials in vascular stents and other biomedical applications and has suitable comprehensive mechanical properties, suitable degradation behavior, and good cell compatibility [35]. When Cu is degraded into Cu-based nanoparticles and enters the human body, this alters blood Cu ion concentrations. When designing degradable Cu-based materials, special attention should be paid to balance local ion release with overall systemic Cu concentrations. Achieving this balance will inform future research and aid in the development of advanced Cu alloy materials.

Zn is a promising material for biodegradable metal stents. In multiple in vivo experiments, Zn has shown good blood compatibility, strong anti-atherosclerotic properties, and lack of cytotoxicity to endothelial cells [36]. In our previous study, we also found that serum Zn levels were significantly associated with increased BMD of total spine and total femur as well as an elevated risk of diabetes and CVD/CHD in participants with serum zinc level ≥100 μg/dL [18]. In the human body, Zn and Cu interact in disease development. Zn deficiency and elevated Cu levels may exacerbate microvascular damage in diabetes [37]. A case-control study of 60 participants showed that higher serum Cu levels were associated with an increased risk of hypertension. A negative correlation was observed between blood pressure and serum Zn levels, suggesting that hypertension may be associated with an imbalance in the bioavailability of Zn and Cu [38]. Studies have also found that Zn supplementation, close to the recommended daily intake can interfere with the utilization of Cu and iron and may adversely affect high-density lipoprotein cholesterol concentrations [39]. In a controlled study of 120 patients with RA and 70 osteoarthritis controls, researchers found that the levels of Cu and ceruloplasmin in the plasma of the patients with RA were significantly increased, whereas Zn levels were significantly reduced [40].

Zn can influence disease occurrence through multiple mechanisms.

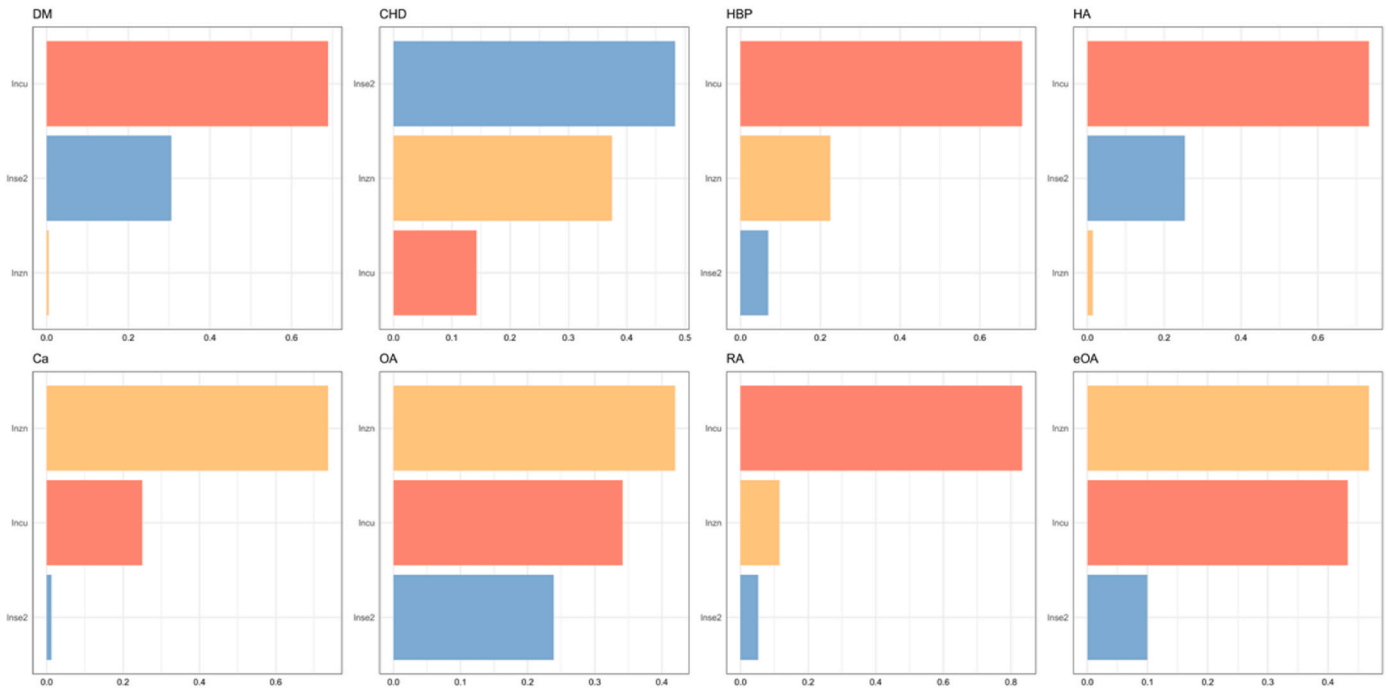

Fig. 3. Estimated weights of heavy metals on the prevalence of different health outcomes in positive direction by WQS.
This model adjusted for all covariates. Cu, copper; Zn, zinc; Se, selenium; DM, diabetes mellitus; CHD, coronary heart disease; HBP, hypertension; HA, heart attack; Ca, cancer; OA, osteoarthritis; RA, rheumatoid arthritis; eOA, early-onset osteoarthritis.

As a redox-inert metal, it can prevent oxidative damage caused by redox-active metals such as iron and Cu through competitive binding [41]. A study on patients with COPD found that Zn concentration in the bronchoalveolar lavage fluid of these patients was significantly reduced and was positively correlated with macrophage phagocytosis [42]. In recent studies, Zn levels in patients with autoimmune diseases were significantly lower than those in healthy controls, suggesting that Zn plays a role in the development of various autoimmune diseases. It can regulate the immune system through multiple molecular mechanisms, including inhibiting Th17 cells and promoting the generation of regulatory T cells [43].

Our study found a significant association between serum Se levels $\geq$122.74 μg/L and the risk of diabetes, as well as between serum Se levels <157.56 μg/L and the risk of heart disease. Se is well known for its antioxidant properties and has recently attracted attention in biomaterial development. However, the therapeutic window for Se is narrow, and its deficiency and excess can have deleterious effects on health. Se deficiency can lead to decreased immune function, decreased cognitive function, and increased mortality; however, excessive Se intake may increase the risk of type 2 diabetes [9]. Most cross-sectional studies have found that serum Se levels are positively correlated with type 2 diabetes because of the complex relationship between Se and selenoproteins, insulin signaling pathways, and carbohydrate/lipid metabolism [44]. In observational studies, blood or nail Se levels were inversely correlated with the risk of CHD; for every 50 % increase in Se concentration, the risk of CHD was reduced by 24 % [45]. Additionally, Se plays a key role in thyroid hormone synthesis and regulates thyroid autoimmune responses [46]. Selenium is involved in various key physiological processes in the human body, including antioxidative stress by binding to selenoproteins, redox regulation, thyroid hormone metabolism, and calcium ion flow [47].

Currently, limited research exists on pure Se metal materials, with most studies focusing on its use in surface coating or in nanoparticle form for antitumor and anti-inflammatory applications [15,48,49]. A major challenge in applying degradable Se-based materials is the utilization of the biological functions of Se while preventing the cytotoxicity caused by its rapid local release. Preliminary experiments have shown that by constructing nanostructures or employing coating technologies, the controlled release of Se ions can be achieved, helping maintain the serum concentrations within a safe and protective range [50]. Based on the threshold data from our study, introducing trace Se elements into future alloy system designs or using surface-coating techniques to regulate Se ion release within specific parameter windows during degradation will be a key research direction.

Beyond the impact of individual serum metal concentrations on human health, our specific research on compound metal mixtures found that serum mixtures containing Cu, Se, and Zn are significantly positively correlated with the risk of diabetes, hypertension, and heart disease. Insufficient research has been conducted on the synergistic effects of multiple metals on alloy systems [51,52]. When designing ternary alloys, balancing the dissolution behavior of each component in the material and its impact on the local cellular environment is necessary. During material degradation, it is essential to maintain the overall mechanical stability and dynamically regulate ion release rates, ensuring that the metal concentration in the serum is within a safe range to preserve material functionality and avoid health risks.

In healthcare settings, medical practitioners can establish risk classification systems for patients based on specific blood metal concentration thresholds. As an example, conducting preoperative assessments for individuals with serum Cu $\geq$ 99.48 μg/dL or Se < 157.56 μg/L could inform decisions regarding appropriate implant options or interventions during the perioperative period [53]. Moreover, integrated monitoring systems combining blood metal concentration thresholds with patient outcomes could be developed for individualized postoperative care. Future investigations should examine how continuous monitoring of serum Cu, Zn, and Se levels can guide real-time adjustments to nutritional supplements or metal chelation treatments [54,55].

Despite these advantages and health implications, the synergistic effects of Cu-Zn-Se combinations in medical materials remain largely unexplored. Understanding their combined effects on human health is crucial for the development of next-generation biomaterials [56]. Fig. 4 illustrates the influence of serum Cu, Zn, and Se, both individually and in

Table 4
Summary of single metal effects and metal mixture interactions on health outcomes using Bayesian Kernel Machine Regression analysis.

Outcomes	Cu	Zn	Se	Overall mixture
Diabetes	No main effect	Suggestive evidence of interaction with Cu when Se is at the median	Significant positive effect when Cu and Zn above 50th percentile; suggestive evidence of interaction with Cu when Zn is at the median	Significant positive effect
CHD	No main effect	Suggestive evidence of interaction with Cu when Se is at the median	Suggestive evidence of interaction with Cu when Zn is at the median	Not significant
Hypertension	Significant positive main effect; suggestive evidence of interaction with Se when Zn is at the median	Suggestive evidence of interaction with Se when Cu is at the median	Suggestive positive main effect	Significant positive effect
Heart attack	Suggestive evidence of interaction with Zn and Se	Suggestive evidence of interaction with Cu and Se	Suggestive evidence of interaction with Cu and Zn	Significant positive effect
Cancer	No main effect	Suggestive evidence of interaction with Cu when Se is at the median	Significant negative effect when Cu and Zn under 50th percentile; suggestive evidence of interaction with Cu when Zn is at the median	Suggestive negative effect
OA	No main effect	Significant positive effect when Cu and Se above 50th percentile	Suggestive negative effect when Cu and Se under 50th percentile; suggestive evidence of interaction with Zn when Cu is at the median	Suggestive positive effect
RA	No main effect	Suggestive evidence of interaction with Cu when Se is at the median	Suggestive evidence of interaction with Cu when Zn is at the median	Suggestive negative effect
early-onset OA	No main effect	Suggestive evidence of interaction with Se when Cu is at the median	Suggestive negative effect when Cu and Zn under 50th percentile	Suggestive negative effect

Cu, copper; Zn, zinc; Se, selenium; Pb, lead; Mn, manganese; Cd, cadmium; CHD, coronary heart disease; OA, osteoarthritis; RA, rheumatoid arthritis.

combination, on human health, offering insights for future research on biodegradable biomaterials.

5. Limitations

This study had some limitations that should be considered when interpreting the findings. First, the cross-sectional nature of the NHANES data precludes causal inferences and the establishment of temporal relationships between metal exposure and health outcomes. Although the NHANES database is highly credible in terms of sampling design and quality control, and its results are highly generalizable to the overall U. S. population, our findings can provide preliminary signals and help rapidly screen for potential risk or beneficial factors. However, noting that the results of this cross-sectional study represent associations is important, and future prospective cohort studies or randomized controlled trials are needed to further validate potential causal relationships.

Second, the NHANES database cannot directly simulate the release scenario of implanted materials, and a single serum measurement may not fully reflect long-term metal exposure patterns, particularly for metals with different half-lives. This study did not directly measure the impact of implant corrosion products on the human body but instead evaluated the potential effects of serum ion concentration levels on immunity and oxidative stress. We recommend designing appropriate in vivo and in vitro experiments using the corresponding composite metal-releasing implants in animal or cell models to directly simulate the impact of ion concentration thresholds at different degradation stages. Our findings provide a reference framework for observing the association between long-term human exposure and health risks and highlight the need to consider potential safety thresholds and bioactivity in future alloy research.

Finally, in cross-sectional studies using the NHANES database, disease status was self-reported, which may have introduced recall bias and misclassification. As an exploratory study, the risk of Type I errors in multiple comparisons exists. The lack of strict multiple comparison corrections may have inflated the statistical significance of some associations. Although methods such as WQS and BKMR mitigate this risk, future research is recommended to validate the current findings using larger sample sizes and more rigorous statistical correction methods. Although our analysis considered several confounding factors, we cannot rule out residual confounding factors due to unmeasured variables. The focus of this study on adult populations may limit its generalizability to other age groups. In addition, the complex survey design and large amounts of missing data reduced sample sizes in some analyses, potentially affecting the statistical power. We recommend repeating the validation in future multiethnic, multiregional, and prospectively designed cohorts using other external databases to minimize the influence of potential confounding factors and enhance the generalizability of the research findings.

6. Implications

This study revealed both individual and overall associations between serum Cu, Se, and Zn concentrations and various health outcomes, highlighting the potential of biodegradable metal-based materials in medical applications.

The study aimed to identify correlations between variables and explore thresholds using traditional regression research methods to enhance result clarity and interpretability. However, with the increase in NHANES data and other database variables, machine learning can serve as a powerful tool to uncover more complex metal interaction patterns, offering more precise support for customized alloy design and clinical interventions. Under high-dimensional data or diverse population characteristics, machine learning algorithms (such as random forests, XGBoost, and deep learning) can detect nonlinear and interaction effects more effectively and enhance predictive accuracy [57–59].

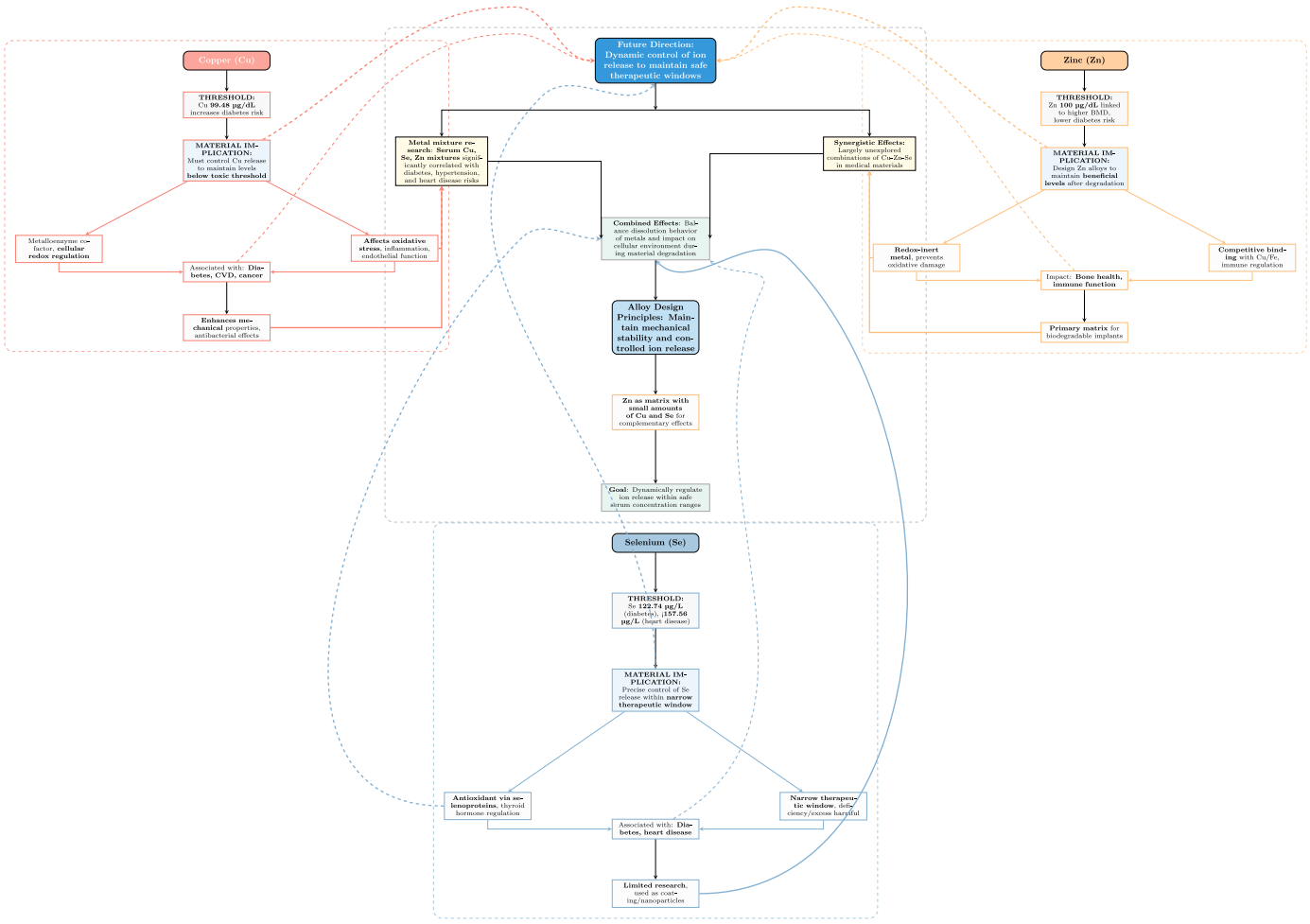

Fig. 4. Comprehensive mechanism of Copper, Zinc and Selenium interactions in health, disease development and implications for biomaterial design.

Future studies should utilize machine learning models to screen for the most significant metal exposures or baseline covariates for a more precise population classification or disease prediction.

Based on our research findings, and considering the complexity of the in vivo environment, we recommend introducing long-term in vivo experiments and highly simulated in vitro dynamic simulation devices for future material design and performance evaluation to evaluate material performance under real physiological conditions, ensuring that composite metal implants are maintained within a safe and effective range throughout the degradation process. We recommend using microfluidic dynamic release models, animal experiments, and real-time monitoring techniques (such as ICP-MS) to develop mathematical models of degradation behavior and serum metal concentrations in the future [60,61].

The construction of multiscale prediction models aids in forecasting the ion release of materials in vivo in the early stages of material design and in the design of safe and effective biodegradable metal implants. Furthermore, a ternary phase diagram of the Cu-Se-Zn system was created to clarify the interactions between the components and their stability in different environments. By systematically adjusting the component ratios, alloy systems can achieve an optimal balance in terms of mechanical properties, degradation rate, and ion release [62,63].

Material science and clinical epidemiology can be combined to explore the quantitative relationship between abnormal changes in specific indicators (such as Cu, Zn, and Se concentrations) and disease risk through large-scale serum metal detection data analyses [54,64]. In addition, the thresholds of composite biomaterials in other disease models and interactions between serum metals can help develop composite biomaterials for various disease applications. This type of interdisciplinary research will provide data to support the clinical application of biodegradable materials in reducing disease risk and will offer a theoretical foundation for "material-biological metal homeostasis regulation" in future medical programs.

7. Conclusions

Serum Cu levels are significantly associated with the risk of diabetes in participants with serum Cu levels ≥99.48 μg/dL. Serum Se levels are significantly associated with the risk of diabetes among participants with serum Se levels ≥122.74 μg/L and with the risk of heart attack among participants with serum Se levels <157.56 μg/L. Serum metal mixtures containing Cu, Se, and Zn are significantly and positively associated with the risk of diabetes, hypertension, and heart attack. Future research should focus on developing targeted interventions, exploring precise molecular mechanisms, and investigating the combined effects of biodegradable multi-metal alloy materials on health outcomes.

CRediT authorship contribution statement

Yufei Wang: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yiwen Sun:** Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Tianyang Jie:** Writing – original draft, Visualization, Data curation. **Minqi Wang:** Methodology, Data curation. **Shutao Zhang:**

Data curation, Methodology, Validation. **Hongtao Yang:** Methodology, Data curation. **Weiyan Jian:** Supervision, Validation. **Dai Dai:** Validation, Supervision, Methodology. **Ruida Xu:** Writing – review & editing, Validation, Supervision, Project administration, Conceptualization. **Bing Yue:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Xinhua Qu:** Writing – review & editing, Validation, Resources, Project administration, Funding acquisition, Conceptualization.

Data sharing

All NHANES data are available at https://wwwn.cdc.gov/nchs/nhanes/.

Data and materials availability

All data needed to evaluate the conclusions in the paper are present in the paper. Additional data related to this paper may be requested from the authors.

Provenance and peer review

Not commissioned; externally peer reviewed.

Ethical approval and consent to participate

This study used publicly available data from the National Health and Nutrition Examination Survey (NHANES), conducted by the U.S. Centers for Disease Control and Prevention (CDC). Data collection for NHANES was approved by the Ethics Review Board (ERB) of the National Center for Health Statistics (NCHS) under protocol #2011-17 for the 2011–2018 cycle, and all participant information was anonymized before public release (https://www.cdc.gov/nchs/nhanes/about/erb.html#print).

Because our study relies solely on de-identified secondary data and does not involve direct participant contact, no additional ethical review or informed consent was required.

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Declaration of competing interest

Xinhua Qu is an early career editorial board member for Bioactive Materials and was not involved in the editorial review or the decision to publish this article. The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioactmat.2025.04.004.

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