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Associations of essential trace elements with epigenetic aging indicators and the potential mediating role of inflammation

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ABSTRACT

Background: Essential trace elements (ETEs) play essential roles in vital functions, but their effects on epigenetic aging remain poorly understood.

Objectives: This study aimed to investigate the associations of ETEs with four epigenetic aging indicators and assess the potential mediating role of inflammation.

Methods: We recruited 93 individuals from hospitals between October 2018 and August 2019. Plasma levels of cobalt, copper, iron, manganese, molybdenum, selenium, and zinc were measured by ICP-MS, and leukocyte DNA methylation levels were measured using Illumina MethylationEPIC beadchip. Linear regression was used to estimate the association between seven plasma ETEs and epigenetic aging indicators. Weighted quantile sum (WQS) regression and Bayesian kernel machine regression (BKMR) models were used to evaluate the effect of ETEs mixtures. Inflammatory status was assessed using four systemic inflammation indices (neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), and systemic immune-inflammation index (SII)) and three cytokines (IL-4, IL-6, and IL-13). Mediation analysis was performed to explore the role of inflammation in the above associations.

Results: Plasma Se levels were significantly negatively associated with DunedinPACE, whereas Cu levels were significantly positively associated with it. Both WQS regression and BKMR models suggested that Se and Cu dominate the effect of the ETEs mixture. MLR and interleukin 6 were significantly and positively associated with DunedinPACE. Further mediation analysis indicated that inflammation partially mediated the association between ETEs and DunedinPACE.

Discussion: Plasma Se and Cu levels are closely associated to epigenetic aging, and inflammation might be a potential mechanism underlying this relationship. These findings contribute to the prevention of health hazards associated with population aging.

1. Introduction

Essential trace elements (ETEs) are defined as the basic elements of life [1]. Although ETEs are in small amounts in the human body, they play an integral role in maintaining human health, such as acting as cofactors for antioxidant enzymes [2,3]. On the other hand, aging is a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death, reflecting the overall health status of individuals [4,5]. As the global population ages at a progressively faster rate, identifying the key factors of biological aging and intervening in them to achieve healthy aging have become an important issue for public health [6]. Previous studies found complex relationships between ETEs and multiple diseases such as cardiovascular disease, neurodegenerative disease, and cancer [7–9]. Given this, these ETEs might also have a wide range of effects on the aging process, in addition to their specific effects on diseases.

Currently, algorithms using blood DNA methylation data are powerful tools for quantifying biological aging, often referred to as epigenetic clocks. PhenoAge [10] and GrimAge [11] are the second epigenetic clocks that use scores based on blood chemical markers and

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plasma protein markers, respectively, to predict risk of death and further fit the scores with DNA methylation data. DunedinPoAm (Dunedin Pace of Aging methylation) is an algorithm designed to capture the rate of aging, which is trained on changes in biomarkers and health indicators in individuals of the same actual age over a 12-year period [12]. The algorithm was subsequently updated as DunedinPACE (Dunedin Pace of Aging Calculated from the Epigenome) [13]. It measured age-related changes in 20 biomarkers in the same population over a 19-year observation period and included only CpG sites with high technical reliability. These epigenetic aging indicators based on biological age have been shown to strongly predict the onset of all-cause mortality and multiple aging-related diseases [14,15].

Limited studies suggest potential associations between some ETEs and epigenetic aging indicators. Results from the Normative Ageing Study showed that Iron (Fe) and Zinc (Zn) levels measured using air samplers are not associated with AgeAccel Pheno in elderly men [16]. Recently, a cross-sectional study investigated the relationship between 18 plasma metals/metalloids and epigenetic aging in 276 Chinese elderly people and found a protective effect of high plasma levels of Cobalt (Co) and Zn on AgeAccel Hovarth, and Zn on AgeAccel Grim [17]. In contrast, in an Amerindian population, Zn was found to be positively associated with biological senescence association (AgeAccel Pheno, AgeAccel Grim, DunedinPACE) [18]. The associations between ETEs and epigenetic aging have not been well evaluated. At the same time, given that humans are always co-exposed to multiple ETEs, there is a need for efforts to explore new evidence on the association between mixtures of ETEs and epigenetic aging.

Inflammation is thought to be "common soil" for the development of many chronic diseases [4,19,20], and one of hallmark of aging [4]. Meantime, some evidence suggests that ETEs are strongly associated with systemic inflammatory status [21–23]. Therefore, inflammation might play a role in the association between ETEs and epigenetic aging.

Accordingly, we conducted the present study to investigate the associations of seven ETEs including plasma Co, copper (Cu), Fe, manganese (Mn), molybdenum (Mo), Se, and Zn with epigenetic aging indicators and potential mixed effects of ETEs on epigenetic aging. Our second objective was to explore the associations between ETEs and indicators of inflammation, and whether inflammation is a potential mediator between ETEs and epigenetic aging.

2. Methods

2.1. Study population

From October 2018 to August 2019, 114 adult volunteers were recruited from Shiyan Renmin Hospital in Hubei Province [24]. They had elective surgery for benign or cosmetic plastic surgery needs, WBC count less than 10×10^9/L, and no serious systemic diseases such as malignancy, autoimmune diseases, heart or kidney failure. Finally, we measured DNA methylation and plasma ETEs levels in 98 participants who provided blood samples and retained 93 samples in the final analysis after quality control (See Fig. S1 for details).

Ethical approvals of this study were obtained from the Medical Ethics Committee of the School of Public Health, Tongji Medical College, Huazhong University of Science and Technology. All participants provided written informed consent.

2.2. Assessment of DNA methylation and epigenetic ages

The epigenome-wide DNA methylation levels were measured using Illumina Human MethylationEPIC BeadChip 850K (Illumina Inc, USA). Raw methylation IDAT files were processed using the "minfi" package and "wateRmelon" in R. The detail of the QC process has been described in our previous study [24]. We normalized the data using the "preprocessNoob" function [25] and the "BMIQ" function [26]. Batch effects are corrected using the ComBat method [27]. After quality control, 94 participants had available DNA methylation data.

PhenoAge and GrimAge are calculated on the online calculator (https://dnamage.genetics.ucla.edu/home). Acceleration of epigenetic ages is the difference between each epigenetic age and the actual age, determined in the form of residuals. Specifically, the data were fitted using a general linear regression model with epigenetic age as the dependent variable and actual age as the independent variable, and residuals were calculated. DunedinPoAm and DunedinPACE are quantifications of the rate of aging and their values reflect the increase in biological age for each year of actual age. DunedinPoAm is calculated using the "DunedinPoAm38" package in R (https://github.com/danbelsky/DunedinPoAm38), and DunedinPACE is calculated using the "DunedinPACE" package (https://github.com/danbelsky/DunedinPACE). More information on the four epigenetic age acceleration indicators were presented in the supplementary document (**Text S1**).

2.3. Determining the concentration of ETEs

Plasma concentrations of seven ETEs (Co, Cu, Fe, Mn, Mo, Se, and Zn) were measured using ICP-MS. The detailed method is consistent with that described in our previous studies [28,29]. Certified reference agents (ClinChek human plasma controls for trace elements no. 8883 and 8884, Recipe, Munich, Germany) and standard reference materials 1640a (Trace Elements in Natural Water, National Institute of Standards and Technology, Gaithersburg, MD) were used for quality control. Table S1 shows the concentration distribution and LOD of the 7 plasma ETEs. Of the 94 individuals with DNA methylation data, 93 had available plasma for testing of ETEs. The detection rate for all 7 ETEs was 100%.

Blood count measurements and derived Systemic Inflammation Index.

Complete blood counts, including levels of absolute peripheral counts of leukocytes, neutrophils, lymphocytes, monocytes, and platelets, were measured in Shiyan Renmin Hospital of Hubei Province using the biochemical automatic detector. We calculated four Systemic Inflammation Index based on peripheral blood counts as follows: (1) Neutrophil-to-lymphocyte ratio (NLR) = neutrophils/lymphocytes; (2) Platelet-to-lymphocyte ratio (PLR) = platelets/lymphocytes; (3) Monocyte-to-lymphocyte ratio (MLR) = monocytes/lymphocytes; and (4) Systemic immune inflammation index (SII) = (neutrophils × platelets)/lymphocytes. These indicators reflect the overall inflammatory status of the body and are associated with increased risk of cancer and cardiovascular diseases [30,31].

2.4. Measurement of inflammatory cytokines

Plasma cytokines, chemokines, and growth factors were measured using the Bio-Plex Pro Human Cytokine 48-Plex Screening Panel (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Signal intensities were read using the Luminex System with HTF (Luminex 200, Luminex Corp, Austin, Texas, USA), and sample concentrations were calculated using Bio-Plex Manager 6. 0 software (Bio-Rad Laboratories). We chose IL-6 as representative of pro-inflammatory cytokines and IL-4 and IL13 as representative of anti-inflammatory cytokines based on the current knowledge [32–34]. Table S2 shows the concentration distribution and LOD of the 3 inflammatory cytokines.

2.5. Definition of covariates

Trained interviewers administered standard questionnaires to collect information on age, sex (male/female), current smoking (current/former or never), and current drinking (current/former or never). Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively, and body mass index (BMI) was calculated as weight divided by height squared (kg/m^2).

2.6. Statistical analyses

The normality of variables was evaluated by the Shapiro–Wilk test. Basic characteristics of the study participants were presented as means (SD) or median (25th, 75th) according to the data distribution, and categorical variables were shown as frequencies (percentages). Before conducting association analysis, plasma ETEs levels, inflammatory cytokines, and systemic inflammation index were natural log transformed to reduce the effect of skewed data on the model and were further z-score transformed to enable comparison of beta coefficients. Epigenetic aging indicators were only z-transformed because it is normally distributed. In addition, due to the low detection rate of IL-6, we transformed it into a dichotomous variable, i.e. whether it was detected or not.

Spearman's correlation coefficient was used to assess the correlation of plasma ETEs or epigenetic aging indicators. A general linear regression model was used to assess the associations of continuous single ETE with epigenetic aging indicators. The false discovery rate (FDR) corrected *P*-value was calculated using the Benjamini–Hochberg procedure [35].

We also applied weighted quantile sum (WQS) regression to assess the association of plasma ETE mixtures with epigenetic aging indicators, as well as the weights of individual ETE [36]. The weights of the 7 elements range from 0 to 1 and the sum is 1. We used two models, assuming a positive and negative association for the overall effect of the mixture, respectively. WQS regression is performed using R package "gWQS". The number of bootstrap samples used in the parameter estimation was set to 1000. The percentage of the data set used to validate the model was 50%.Then, bayesian kernel machine regression (BKMR) analysis was performed using the R package "bkmr" to assess potential non-linear exposure-response and interaction relationships between ETEs and epigenetic aging indicators [37]. The BKMR estimates were generated after 25,000 iterations of the Markov chain Monte Carlo.

Afterward, we used general linear regression models to assess the association of continuous plasma levels of 7 elements with indicators of inflammation, except for IL-6 which was assessed using logistic regression models as it was transformed into a dichotomous variable. General linear regression models were used to assess the association of inflammatory indicators with DunedinPACE. Finally, mediation analysis was performed using the R package "mediation" to assess the mediating role of IL-6 and MLR in the association of plasma ETEs with DunedinPACE. Related statistical results were obtained by quasi-Bayesian Monte Carlo simulation for 3000 times.

All regression models were adjusted for gender (male/female), BMI (continuous), smoking status (current/former or never), and alcohol consumption status (current/former or never). Age was additionally adjusted in the regression models exploring the association of ETEs with inflammatory indicators. A two-side P value < 0.05 was considered to be statistically significant, and all statistical analyses were performed by using R version 4.1.1 (R Foundation for Statistical Computing).

3. Results

3.1. Basic characteristics

This study included 93 participants, of whom 70 (75.27%) were female, the median age of the participants was 48 (44, 52) years, the mean BMI was 24.48 ± 3.10 kg/m^2, and 14 (15.05%) and 18 (19.35%) reported being current smokers and current drinkers respectively (Table 1). The correlations between the 7 plasma ETEs levels were weak, only Fe, Mn, and Mo had a significant correlation (rho from 0.25 to 0.37), and the rest were not correlated (Fig. S2). Two epigenetic ages were highly correlated with chronological age (rho = 0.68 for Pheno Age and 0.81 for Grim Age), while four epigenetic aging indicators, including AgeAccel Pheno, AgeAccel Grim, DunedinPoAm, and DunedinPACE, were not correlated with chronological age (Fig. S3).

Table 1
Characteristics of the study participants (n = 93).

Variables	Statistical Description
Age, years	48 (44, 52)
Female, n (%)	70 (75.27)
Body mass index (BMI, kg/m^2)	24.48 ± 3.10
Current cigarette smoker, n (%)	14 (15.05)
Current alcohol drinker, n (%)	18 (19.35)
Epigenetic ages	
DNAme PhenoAge, years	44.65 (39.72, 51.08)
AgeAccel Pheno, years	0.03 ± 7.13
DNAme GrimAge, years	55.20 (52.05, 60.75)
AgeAccel Grim, years	−0.39 ± 3.83
DunedinPoAm	1.02 ± 0.12
DunedinPACE	1.17 (1.10, 1.26)
Systemic Inflammation Index	
Lymphocyte counts,10^9/L	1.37 (1.06, 1.62)
Neutrophil counts,10^9/L	3.66 (2.68, 5.16)
Monocyte counts,10^9/L	0.41 (0.32, 0.54)
Platelet counts,10^9/L	230 (188, 293)
Neutrophil to lymphocyte ratio (NLR)	2.68 (1.74, 4.05)
Monocyte to lymphocyte ratio (MLR)	0.30 (0.21, 0.41)
Platelet to lymphocyte ratio (PLR)	170.95 (129.09, 194.88)
Systemic inflammation response index (SII)	613.30 (393.30, 970.10)
Inflammatory cytokines	
Lnterleukin 4 (IL-4, pg/mL)	0.78 (0.61, 1.03)
Lnterleukin 6[a] (IL-6, pg/mL)	0.06 (0.06, 2.91)
Lnterleukin 13 (IL-13, pg/mL)	1.98 (1.16, 3.98)

Note: Continuous variables were presented as means (SD) or median (25th, 75th) according to the data distribution and categorical variables were shown as frequencies (percentages).
[a] interleukin 6 was only detected in 39 individuals.

3.2. Associations between single ETE and epigenetic aging

Fig. 1 and Table S3 shows associations of single ETE with four epigenetic aging indicators. Each 1-SD increment in the log-transformed concentrations of plasma Co and plasma Cu was associated with a 0.24 (0.02, 0.45) and 0.31 (0.11, 0.52) unit increase in Z-transformed DunedinPoAm and DunedinPACE, respectively. Each 1-SD increment in the log-transformed concentration of plasma Mn was associated with a −0.32 (−0.63, −0.00) unit decrease in Z-transformed DunedinPoAm. Each 1-SD increment in plasma Se log-transformed concentrations was associated with a −0.21 (−0.42, −0.01) and −0.31 (−0.51, −0.11) unit decrease in Z-transformed AgeAccel Pheno and DunedinPACE, respectively (all P values < 0.05). After multiple corrections, only plasma Cu and Se were significantly associated with DunedinPACE (FDR <0.05). We did not find any plasma ETEs associated with AgeAccel Grim.

3.3. WQSR analysis of associations between ETE mixtures and epigenetic aging

In the WQS regression assuming a positive effect, we observed that the positive association between a mixture of 7 ETEs and DunedinPACE reached nominal significance, and plasma Cu weighted more than 50% (Fig. 2A, $P = 0.048$). Assuming negative effects, we did not observe an association between the mixture of 7 ETEs and DunedinPACE, and plasma Se weighted more than 50% (Fig. 2B, $P = 0.291$). The associations between the mixture and other epigenetic aging indicators was not significant, regardless of whether the direction of the association was set to be positive or negative (Fig. S4 and Fig. S5).

3.4. BKMR analysis of associations between ETEs mixtures and epigenetic aging

Next, we further explored the association between ETE mixtures and epigenetic aging indicators using the BKMR model. In the BKMR model, we did not observe associations between the seven ETE mixtures and the four epigenetic aging indicators (Fig. 2C, Fig. S6). Subsequently, we

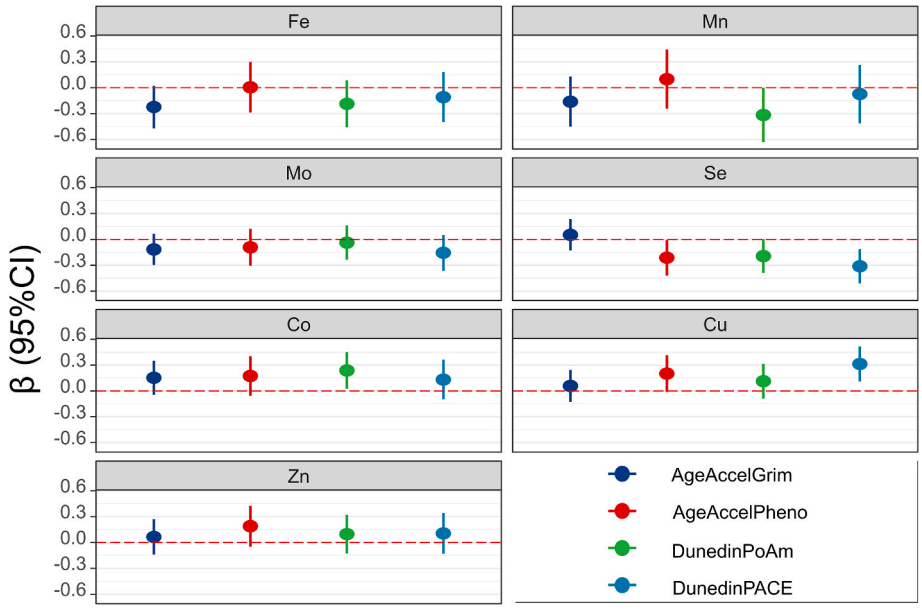

Fig. 1. Associations between single plasma ETE and epigenetic aging (N = 93). Linear regression models were adjusted for gender (male/female), BMI (continuous), smoking status (current/former or never), drinking status (current/former or never). β means the change of the standardized epigenetic aging indicators for each 1 SD increase in natural log-transformed plasma ETE concentration. The value of β at the red dashed line is 0. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

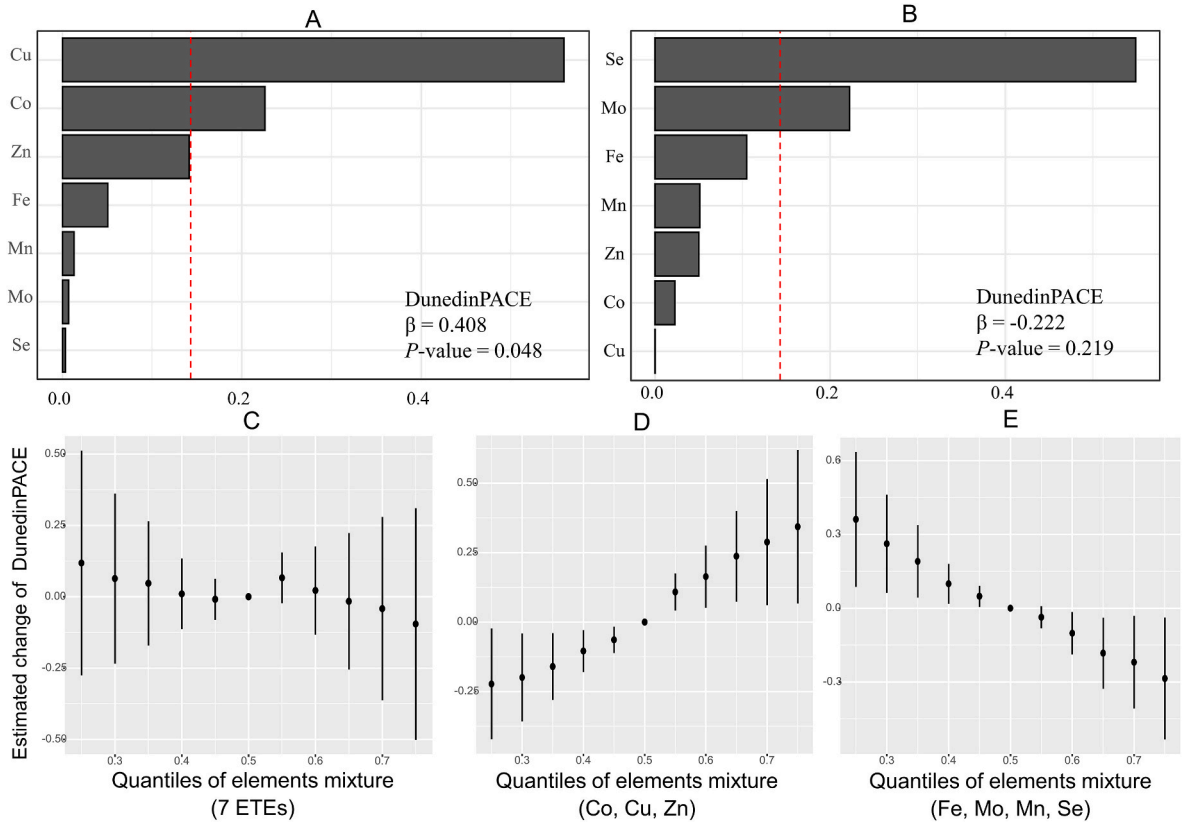

Fig. 2. Weighted Quantile Sum regression for essential trace elements associated with DunedinPACE, and BKMR analysis for the associations of ETEs mixture with DunedinPACE. A: regression index weights of essential trace elements in WQS models (Coefficient set to positive). B: regression index weights of essential trace elements in WQS models (Coefficient set to negative). C: Overall effect of the seven ETEs mixtures: estimates (black dotted) and 95%CI (error bars) when all elements at particular percentiles were compared with their 50th percentiles. D: Overall effect of the Co, Cu, and Zn mixtures. E: Overall effect of the Fe, Mo, Mn and Se mixtures. Models were adjusted for gender (male/female), BMI (continuous), smoking status (current/former or never), and drinking status (current/former or never). The red dashed line represents the average weights of seven elements (1/7). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

divided the seven ETEs into two groups (Co, Cu and Zn, which showed a positive trend in the linear regression model and the WQS model, and Fe, Mn, Mo and Se, which showed a negative trend in the linear regression model and the WQS model, respectively). We found that mixtures of Cobalt, Copper and Zinc were significantly positively correlated with DunedinPACE (Fig. 2D). In contrast, mixtures of iron, molybdenum, manganese, and selenium showed a significant negative correlation with DunedinPACE (Fig. 2E). Meanwhile, we found similar results in the BKMR model with AgeAccel Pheno, AgeAccel Grim, and DunedinPoAm, although not as significant as with DunedinPACE (Fig. S6). We observed negative association of plasma Se and Mo with DunedinPACE, as well as a positive association for Cu, when all other elements were in the 50th percentile (Fig. S7A). Also, we found that there was an interaction between plasma Cu and Se (Fig. S7B). When other elements levels were fixed at their 25th, 50th, and 75th levels, respectively, we observed marginal significant negative associations between plasma Se and DunedinPACE while positive association of plasma Cu with DunedinPACE (Fig. S7C).

3.5. Associations between single ETE and inflammatory indicators

Similar to the association between plasma ETEs and epigenetic aging indicators, plasma levels of Cu, Co, and Zn were positively associated with pro-inflammatory markers and negatively associated with anti-inflammatory markers, whereas plasma levels of Fe, Mn, Mo, and Se had the opposite effect (Fig. 3, Table S4, Table S5). We found a positive association between plasma Co levels and IL-6 levels, while increased plasma Fe, Mo, and Se levels were associated with a decrease in IL-6 levels. For other inflammatory indicators, negative association of plasma Fe and Mo levels with MLR and positive association of plasma Co levels with PLR remained significant after multiple corrections. Plasma Co was negatively associated with anti-inflammatory indicator (IL-13) and Se was positively associated with IL-13 (FDR <0.05).

3.6. Mediation effects of inflammatory indicator in the associations between ETEs and DunedinPACE

As shown in Table S6, the association of MLR and IL-6 with DunedinPACE was statistically significant. For each 1-SD increment in the log-transformed levels of MLR, DunedinPACE increased by 0.31 (0.10, 0.51). Compared with the undetected plasma IL-6 group, those with IL-6 above the LOD had increased Dunedin PACE by 0.62 (0.22, 1.01). We then evaluated the mediating role of MLR and IL-6 in the association between 7 plasma ETEs and DunedinPACE. We found that MLR partially mediated the negative association of plasma Se with DunedinPACE (mediation proportion was 16.6% (0.2, 54.0%), *P*-value = 0.048). In addition, although the total effect was not significant, we found significant indirect effect of MLR in the associations between plasma Co, Fe, and Mo and DunedinPACE, and significant indirect effect of IL-6 in the associations between plasma Fe and DunedinPACE (Table 2).

4. Discussion

In this study, we explored the associations between plasma ETEs and four epigenetic aging indicators. Our study indicated that plasma Se were significantly negatively while Cu were significantly positively associated with DunedinPACE. In addition, we found that inflammation may partially mediate the association between ETEs and epigenetic aging.

Biological age reflects a person's physiological state and function, and is a better indicator of the stage a person is at in life than chronological age, as well as a better indicator of a person's health characteristics than a single disease state [38,39]. Studies shows that epigenetic age based on DNA methylation is the most promising predictor of biological age [38,40]. Many longitudinal cohort studies demonstrated the ability of epigenetic aging indicators to predict morbidity and mortality, however, factors influencing epigenetic aging indicators still remain to be investigated. Our study suggests that ETEs are clearly one of the important factors influencing epigenetic aging. Our study also found

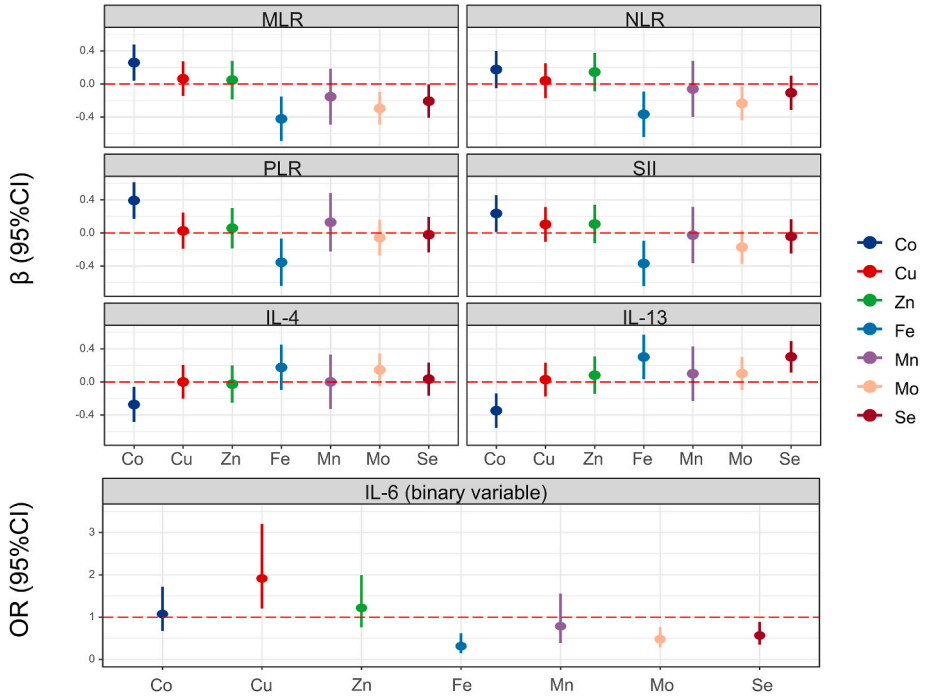

Fig. 3. Associations between single plasma ETE and inflammation indicators (N = 93). Note: Systemic Inflammation Index: MLR, NLR, PLR, SII; Anti-inflammatory cytokines: IL-4, IL-13; Pro-inflammatory cytokine: IL-6. IL-6 was included in a logistic regression model based on whether it was detected as a categorical variable, while the remaining inflammatory indicators were included in the linear regression model as z-transformed continuous variables. Models were adjusted for Age (continuous), gender (male/female), BMI (continuous), smoking status (current/former or never), and drinking status (current/former or never).

Table 2
Mediation analysis of inflammation indicators on the association of plasma ETEs with DunedinPACE.

Exposure	Mediator	Mediating effects (ACME)		Direct effect (ADE)			
		β (95% CI)	*P*-value	β (95% CI)	*P*-value	Mediation proportion % (95% CI)	*P*-value
Co	IL-6	0.009 (−0.105, 0.130)	0.880	0.123 (−0.099, 0.340)	0.260		
Cu	IL-6	0.070 (−0.018, 0.200)	0.136	0.242 (0.055, 0.420)	0.014	20.3% (−10.1%, 64.0%)	0.130
Fe	IL-6	**−0.135 (-0.314, 0.000)**	**0.040**	0.043 (−0.303, 0.380)	0.819		
Mn	IL-6	−0.030 (−0.181, 0.110)	0.630	−0.042 (−0.359, 0.270)	0.790		
Mo	IL-6	−0.087 (−0.228, 0.010)	0.089	−0.061 (−0.281, 0.150)	0.587		
Se	IL-6	−0.059 (−0.179, 0.030)	0.185	−0.248 (−0.452, −0.030)	0.025	18.0% (−15.3%, 64.0%)	0.187
Zn	IL-6	0.027 (−0.093, 0.160)	0.610	0.074 (−0.154, 0.290)	0.490		
Co	MLR	**0.072 (0.008, 0.160)**	**0.023**	0.059 (−0.159, 0.280)	0.596		
Cu	MLR	0.017 (−0.042, 0.080)	0.564	0.295 (0.114, 0.480)	0.002	5.1% (−21.2%, 27.0%)	0.563
Fe	MLR	**−0.135 (-0.283, -0.030)**	**0.005**	0.023 (−0.279, 0.320)	0.895		
Mn	MLR	−0.057 (−0.190, 0.060)	0.320	−0.017 (−0.309, 0.280)	0.910		
Mo	MLR	**−0.079 (-0.179, -0.010)**	**0.012**	−0.081 (−0.301, 0.150)	0.462		
Se	MLR	**−0.055 (-0.135, -0.004)**	**0.040**	−0.259 (−0.468, −0.050)	0.019	**16.6% (0.2%, 54.0%)**	**0.048**
Zn	MLR	0.012 (−0.068, 0.100)	0.760	0.089 (−0.130, 0.320)	0.430		

The estimate of average mediation effects (ACME), the estimate of the average direct effects (ADE) and the proportion of mediation (ACME/ADE + ACME). Models were adjusted for gender (male/female), BMI (continuous), smoking status (current/former or never), drinking status (current/former or never). The mediation ratio is calculated only in associations where the total effect is significant.

that DunedinPACE may be the most sensitive indicator of exogenous environmental effects compared to other epigenetic aging indicators. This is consistent with some previous studies. A study found that DunedinPACE was strongly associated with more health outcomes than other epigenetic aging indicators in Taiwanese biobanking participants [15]. Another study also reported that DunedinPACE, but not AgeAccel Pheno, and AgeAccel Grim, was associated with multiple measures of cognitive aging and dementia [41]. A recent RCT study showed that caloric restriction slowed the pace of aging in adults as measured by the DunedinPACE algorithm, not PhenoAge and GrimAge [42]. The value of DunedinPACE as an indicator of biological aging reflecting the effect of ETEs still needs further confirmation.

Previous prospective studies have highlighted strong association between maintaining adequate Se levels and reduced all-cause mortality [43] and cardiovascular disease incidence [44]. In the present study population, the median of plasma Se level was 46.75 μg/L, which was significantly lower than that of adults in the UK 2001 NDNS population (85 μg/L in plasma, representing status in Europe) [45], and the US 2003–04 NHANES population (125.6 μg/L in serum, representing status in North America) [43]. In the present population with relatively low levels of Se, we observed a significant negative association between plasma Se levels and DunedinPACE (FDR <0.05). Also, we found that Se dominated the negative association between ETE mixture and DunedinPACE. Few studies explored the association of Se with epigenetic aging. A cross-sectional study with 276 Chinese elderly people did not find any association between blood Se levels and four indicators of accelerated epigenetic age (AgeAccel Hovarth, AgeAccel Hunnum, AgeAccel Pheno, AgeAccel Grim) [17]. This may be due to the higher Se levels in this population (median: 161 μg/L of Se in blood). In American Indian populations, urinary selenium levels (median 49.1 mg/g creatinine) were negatively associated with biological aging, although not at a statistically significant level [18]. Our study adds to the preliminary evidence of the beneficial effects of maintaining high Se levels on delaying aging, at least in populations with relatively low levels of Se.

In parallel, we observed a linear positive association between plasma Cu and DunedinPACE and found that Cu dominated the positive association between seven ETEs and DunedinPACE. Cu is an ETE for life and is important in various enzymatic reactions [1]. However, epidemiological evidence suggests that high Cu levels may be hazardous to human health [46]. Our group also reported positive associations between plasma Cu and all-cause and cardiovascular mortality [47], incidence of acute coronary syndrome [48], and serum C-reactive protein levels [49]. The harmful effects of Cu may be due to the ability to displace less competitive metal ions, such as Zn and Fe, from metalloproteins [1]. Only one study investigated the association of Cu with epigenetic aging indicators. This study did not find any association, either positive or negative, between blood Cu levels and the four epigenetic aging indicators (AgeAccel Hovarth, AgeAccel Hunnum, AgeAccel Pheno, AgeAccel Grim) [17]. In the present study DunedinPACE captures potential link between Cu and biological aging, providing preliminary evidence that there is a positive association between plasma Cu levels and DunedinPACE, and that excess Cu may accelerate ageing in humans.

Some evidence indicated that ETEs are strongly associated with systemic inflammatory status [21–23]. Researchers observed that serum Cu levels were strongly correlated with serum TNF-α levels and liver Fe concentrations were correlated with hepatic pro-inflammatory cytokines [50]. Interestingly, in the present study, we found that the associations of ETEs with biological aging and ETEs with inflammation levels showed a similar pattern. Chronic and low-grade inflammation is one of the 7 key pillars of biological aging [51]. Previous studies found that indicators of inflammation independently predict the risk of future death [52]. Studies also indicated associations between inflammatory indicators and epigenetic aging. In a cross-sectional study, age acceleration Hannum was positively associated with IL-6, C-reactive protein (CRP), and tumor necrosis factor α (TNF-α) [53]. In the present study, we also found significant positive associations between DunedinPACE and MLR (an indicator of systemic inflammation derived from blood counts) and IL-6. Mediation analysis found that MLR significantly mediated the negative association between Se and DunedinPACE with a mediation percentage of 16.6%. In addition, although the association of Co, Fe and Mo with DunedinPACE was not significant, the mediation analysis suggested significant indirect mediating effects of MLR on the association between Co, Cu, Fe and Mo with DunedinPACE. Combined with these results, our study suggests that inflammation might be one of the mechanisms by which ETEs contribute to biological aging.

Our study has several strengths. First, we used four epigenetic aging indicators to reflect biological aging and reported associations between Se and Cu and epigenetic aging in adults. Secondly, we used 3 statistical models, including general linear regression model as well as WQSR and BKMR model that considered multiple ETEs mixtures, and found a dominant role for Se and Cu under 3 models. Finally, we explored the role of inflammation in the association between essential micronutrients and biological ageing, providing a valuable opportunity to discover underlying biological mechanisms. However, there are some limitations. Firstly, the study was based on a cross-sectional design. Although the present findings are biologically plausible, confounding factors cannot be excluded. Secondly, our sample size was relatively small and the range of ETEs levels was relatively narrow. Validation of our findings in a large sample study is needed. Third, plasma may not be the best

marker of for all elements, such as Fe [29]. This may be one reason why we did not find an association between Fe and epigenetic aging indicators. Fourth, only three inflammatory cytokines and four systemic inflammation indices were used to indicate inflammation in the present study, which may not fully reflect an individual's inflammatory status, and future studies are warranted to use much more metrics to comprehensively measure inflammation. Fifthly, there were 75.27% females included in the present study, which may limit the generalizability of our findings to the other population. Finally, participants in this study were hospital in-patients with a mean age of 48 years and extrapolation of the results needs to be done with caution, especially as biological aging may be more pronounced in older age. Further validation of the associations between ETEs and biological aging in large prospective cohort studies is warranted in the future. Likewise, the underlying mechanisms remained to be further explored.

In conclusion, the present study showed a significantly negative association between plasma Se and indicators of biological ageing, while a significant positive association was found for plasma Cu. Furthermore, our study reveals that inflammation may play a mediating role in the association between ETEs and biological aging. These findings contribute to a better understanding of the role played by ETEs in the aging process. Public health interventions targeting ETEs will help to mitigate the enormous health as well as economic challenges posed by population aging.

Authors' contributions

XC, MAH contributed to conception and study design, completed data collection and laboratory analyses, performed analyses, interpreted results, and drafted and revised the manuscript critically. YW contributed to study design, completed data collection and laboratory analyses. RXW, CYJ, JA, WYL and JZZ participated in the data collection, laboratory analyses, and manuscript revision. MAH were responsible for obtaining all necessary resources for the study and critically revised all manuscript drafts, including the final version. All authors gave final approval of the version to be published.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2023.102910.

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