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RESEARCH ARTICLE

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Establishing reference intervals for vitamins A and E in Chinese elderly people using liquid chromatography-tandem mass spectrometry

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Abstract

Background: Vitamins A and E play important roles in sustaining life activities and maintaining a good physical condition. However, most people, particularly the elderly, experience micronutrient deficiencies. This study aimed to establish reference intervals (RIs) for vitamins A and E in Chinese elderly people using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.

Methods: A total of 356 apparently healthy individuals aged ≥64 years who underwent health checkups were randomly selected for the study. Vitamin A and E levels were measured using LC-MS/MS. The effect of sex on vitamin A and E levels was evaluated, and RIs were established using a parametric method.

Results: Females showed significantly higher levels of vitamin E than males ($p < 0.05$). However, no significant sex-specific difference was observed with vitamin A levels. The RI for vitamin A in the elderly was 0.283–0.730 mg/L. For vitamin E, the RIs were 4.39–15.63, 4.51–16.14, and 4.41–14.67 mg/L for the total, female, and male participants, respectively. In multiple linear regression, alanine aminotransferase, glutamyl transpeptidase, urea, glucose, and uric acid levels increased with increasing vitamin A levels ($p < 0.05$), and total cholesterol and low-density lipoprotein cholesterol levels increased with increasing vitamin E levels ($p < 0.05$). Direct bilirubin levels decreased with increasing vitamin E levels ($p < 0.05$).

Conclusions: This study established RIs for vitamins A and E in Chinese elderly individuals using an LC-MS/MS method. We also found that females had significantly higher vitamin E levels than males. The findings could provide a scientific basis for interpreting vitamin status in the elderly.

KEYWORDS
LC-MS/MS, reference interval, the elderly, vitamin A, vitamin E

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1 | INTRODUCTION

Vitamins A and E play key roles in sustaining life activities and keeping a good physical condition. The most important and commonly measured form of vitamin A is all-trans-retinol, whereas vitamin E has four forms, α-, β-, γ-, and δ-tocopherol. α-Tocopherol is the most abundant form and accounts for the highest biological activity in humans[1]; thus, it is often used to represent the vitamin E level. Vitamin A improves retinal function (eg, adaptation to dim light) and enhances immune function, reducing the consequences of infectious diseases.[1,2] Vitamin E, on the other hand, functions as an antioxidant and free-radical scavenger, protecting the integrity of unsaturated lipids in the biomembranes of all cells and preserving vitamin A from oxidative destruction.[3] In general, severe or prolonged vitamin A deficiency leads to dry eye (xerophthalmia), which could result in corneal ulcers, scarring, and blindness,[4] and plasma vitamin E deficiency is associated with increased infection, anemia, and stunted growth.[5]

Nowadays, deficiencies in micronutrients including vitamins A and E are a worldwide issue.[6,7] It is estimated that 2 billion people worldwide have micronutrient deficiencies.[8] Therefore, estimating the distribution of vitamin A and E levels in healthy individuals is crucial to assessing whether deficiencies exist. Such estimation is possible with the use of a reference interval (RI), which is defined as the central distribution of values for a certain percentage (usually 95%) of apparently healthy individuals, according to the C28A3 guideline[9] By fitting the original data distribution and adjusting the data distribution through the use of data transformation algorithm, one can establish the distribution range for healthy individuals using a parametric or nonparametric method to provide a basis for clinical decision-making.[9] Establishing RIs for micronutrients can therefore provide a tool for assessing nutritional status.

In 2018, China had 166.58 million people aged >65 years, accounting for 11.9% of the total world population (Available from: http://www.stats.gov.cn/tjsj/tjgb/ndtjgb/). A previous study showed that aging is always accompanied by a general decline in organ functions and loss of muscle mass.[10] Inadequate micronutrient intake is also an issue among the elderly.[11-13] However, a well-established RI for either vitamin A or E in the elderly is still lacking. Thus, it is essential for clinical laboratories to establish RIs in the elderly that are specific to laboratory instruments, reagents, and methods so that clinicians can properly interpret the results.

Therefore, this study aimed to establish RIs for vitamins A and E in Chinese elderly people using an established liquid chromatography-tandem mass spectrometry (LC-MS/MS) method,[14] to provide a reference for evaluating vitamin A and E status. The effects of vitamins A and E on common biochemical analytes were also analyzed using a multifactorial model that included intrinsic factors such as sex and age. Our findings could provide clues for further study on causal relationships between micronutrient deficiencies and related clinical outcomes.

2 | MATERIALS AND METHODS

2.1 | Selection of participants

We randomly selected elderly individuals aged 64–88 years who underwent checkups at Peking Union Medical College Hospital from November 2018 to February 2019. Participants experiencing systemic disease or who were diagnosed with cardiovascular, renal, gastrointestinal, or pulmonary disease or cancer were excluded. Using a statistical method, we also excluded those with outliers or incomplete information. Finally, we enrolled a total of 356 elderly individuals for analysis. Residual serum samples were collected and stored at −80°C until analysis.

2.2 | Ethical approval

This study was approved by the Ethics Committee of Peking Union Medical College Hospital of the Chinese Academy of Medical Sciences. The approval number is S-K1039. Since this study uses the remaining serum of the subjects after examination and all data are privatized, it will not pose any risk to the subjects.

2.3 | Laboratory measurements

Vitamin A and E levels were measured with a previously established LC-MS/MS method.[14] Briefly, 0.05 ml of standard-solution, quality-control serum was mixed with 0.02 ml of internal standards, treated with 0.2 ml ethanol solution, and precipitated with zinc sulfate solution; 0.50 ml hexane was used to extract vitamins A and E. After centrifugation, 0.4 ml of the supernatant was extracted and evaporated by drying in a stream of nitrogen. The dry sample was reconstituted with 0.5 ml methanol-water (80:20) solution. The residuals were analyzed using an API 4000 QTRAP triple quadruple mass spectrometer (Sciex Applied Biosystems, Foster City, CA, USA) with a Waters ACQUITY UPLC system (Waters Corporation, Milford, MA, USA). A Waters ACQUITY UPLC BEH phenyl column (2.1 × 50 mm, 1.7 μm) was used for chromatographic separation. Mobile phase A consisted of water containing 0.1% formic acid, and mobile phase B was methanol. Gradient elution was performed as follows: 0–0.5 min, 60% B; 1.5–2.0 min, 90% B; 2.01–3.0 min, 100% B; and 3.01–4.5 min, 60% B, at a flow rate of 0.4 ml/min. Positive electrospray ionization and multiple reaction monitor modes were used for mass analysis. The multiple reaction monitor channels were as follows: m/z 269.2 → 95.2 [vitamin A], 431.5 → 165.0 [vitamin E], 275.3 → 96.3 ([2H]6-retinol), and 437.5 → 171.3 ([2H]6α-tocopherol). Compared with the reference values for SRM 968e (purchased from the National Institute of Standards and Technology), the accuracy of this method was <5.8% for vitamin A and <5.1% for vitamin E. The procedure also underwent External

Quality Assessments by the College of American Pathologists, and the 2019 results were satisfactory. Vitamin E corrected by cholesterol levels was calculated by vitamin E level (μmol/L) and divided by total cholesterol level in mmol/L.[15]

2.4 | Statistical analysis

We analyzed data using Excel 2016 (Microsoft, Redmond, WA, USA), SPSS 25.0 (IBM Inc., Armonk, NY, USA), and MedCalc 18.116.6 (Mariakerke, Belgium). The normality of data was evaluated using the Shapiro-Wilk test. Normally distributed data are expressed as means and standard deviations, while non-normal data are presented as medians and interquartile distances. The Mann-Whitney *U* test was used to compare differences between males and females. If the data satisfied the assumption of a normal distribution, we used a parametric method to estimate the RI; otherwise, we used a non-parametric method. Tukey's method was used to identify outliers before establishing RIs for vitamins A and E. A multiple linear regression model was used to analyze the effects of vitamins A and E on common biochemical analytes. RIs were also established to observe for significant sex-specific differences. A *p*-value of <0.05 was considered statistically significant.

3 | RESULTS

3.1 | Baseline characteristics

The baseline characteristics of the 356 elderly participants are shown in Table 1. Among the participants, 159 were female (44.6%) and 197 were male (55.4%). The median vitamin A and E levels were 0.483 and 8.710 mg/L, respectively, and the average age was 68 years. Total bilirubin, direct bilirubin, glutamyl transpeptidase, alkaline phosphatase, lactate dehydrogenase, sodium, chloride, uric acid, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels were significantly different between males and females ($p < 0.05$).

3.2 | Effects of sex on vitamin A and E levels

The sex-specific distribution of vitamin A and E levels in the elderly is presented in Figure 1. A significant difference in vitamin E levels was found between males (median [P25-P75]: 7.790 [6.450–9.755] mg/L) and females (9.890 [7.800–12.300] mg/L) ($p < 0.01$). However, we observed no significant sex-specific difference in vitamin A levels (males, 0.491 [0.407–0.581] mg/L, vs. females,

TABLE 1 Baseline characteristics of participants

Characteristic	Unit	Total	Females	Males	*p*
N	–	356	159	197	–
Age	Years	67 (65, 71)	67 (65, 72)	66 (65, 70)	0.290
TP	g/L	73 (71, 75)	73 (71, 75)	73 (70, 75)	0.941
Alb	g/L	45 (44, 47)	45 (44, 47)	45 (44, 47)	0.902
TBil	μmol/L	11.5 (9.3, 14.5)	10.6 (8.6, 12.7)	12.5 (9.7, 16.0)	<0.05
DBil	μmol/L	4.3 (3.6, 5.3)	4.0 (3.4, 4.8)	4.6 (3.7, 5.8)	<0.05
ALT	U/L	18.0 (14.0, 24.0)	17.0 (13.0, 23.5)	18.0 (15.0, 24.0)	0.452
GGT	U/L	20.0 (16.0, 29.0)	18.0 (14.0, 25.0)	21.0 (17.0, 33.0)	<0.05
ALP	U/L	71 (60, 84)	75 (64, 89)	66 (58, 79)	<0.05
AST	U/L	20.0 (17.0, 24.0)	20.0 (17.0, 26.0)	20.0 (17.0, 23.0)	0.234
LD	U/L	188.0 (170.0, 207.0)	194.0 (176.8, 213)	179.0 (168.0, 201.0)	<0.05
Potassium	mmol/L	4.3 (4.1, 4.5)	4.3 (4.1, 4.6)	4.2 (4.1, 4.5)	0.927
Sodium	mmol/L	142 (141, 143)	143 (141, 144)	142 (141, 143)	<0.05
Chloride	mmol/L	101 (99, 103)	102 (100, 103)	100 (99, 102)	<0.05
Urea	mmol/L	4.81 (4.00, 5.63)	4.64 (3.79, 5.64)	4.88 (4.21, 5.63)	0.163
Glu	mmol/L	5.60 (5.20, 6.40)	5.60 (5.10, 6.20)	5.70 (5.20, 6.70)	0.777
UA	μmol/L	305.0 (245.0, 361.0)	264.0 (224.0, 310.0)	336.0 (278.0, 385.8)	<0.05
TC	mmol/L	4.91 (4.05, 5.56)	5.20 (4.49, 5.69)	4.55 (3.82, 5.37)	<0.05
TG	mmol/L	1.39 (0.99, 1.94)	1.39 (1.05, 1.90)	1.36 (0.98, 1.98)	0.915
HDL-C	mmol/L	1.23 (1.05, 1.43)	1.37 (1.18, 1.54)	1.13 (0.99, 1.30)	<0.05
LDL-C	mmol/L	3.18 (2.37, 3.79)	3.36 (2.61, 3.83)	2.96 (2.29, 3.72)	<0.05

Note: Data are expressed as medians with quartiles (25th and 75th).

Abbreviations: Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DBil, direct bilirubin; GGT, glutamyl transpeptidase; Glu, glucose; HDL-C, high-density lipoprotein cholesterol; LD, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; TBil, total bilirubin; TC, total cholesterol; TG, triglycerides; TP, total protein; UA, uric acid.

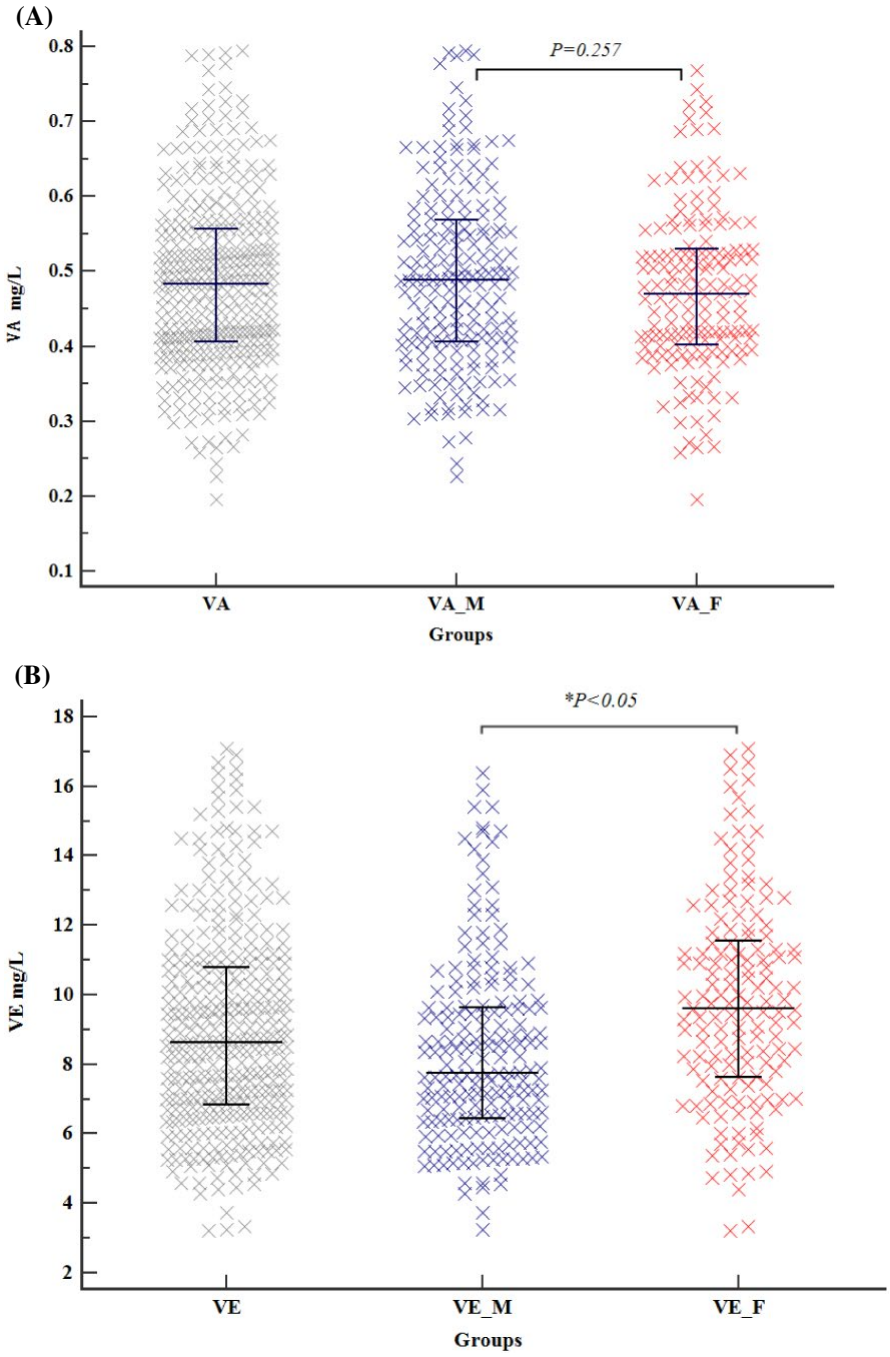

FIGURE 1 Comparison of the differences in (A) vitamin A and (B) vitamin E levels between males and females. VA, vitamin A; VE, vitamin E; M, males; F, females

0.472 [0.403–0.534] mg/L) after using the Mann-Whitney *U* test (p = 0.257). In the multiple linear regression model, we found a significant sex-specific difference in vitamin E levels (p = 0.002).

3.3 | Established RIs for vitamins A and E

Using Tukey's method, we identified the lower limits of vitamin A and E levels as 0.172 and 0.806 mg/L, and the upper limits as 0.796 and 17.116 mg/L, respectively. Through a Box-Cox conversion, the normality of data was improved, and the RIs for vitamin A and vitamin E were established based on the parametric method. Sex-specific RIs were also established (Table 2). Furthermore, the RIs of vitamin E corrected by total cholesterol were 2.62–7.81. There was still a significant difference of vitamin E between females and males after correction for total cholesterol (p = 0.016).

3.4 | Effects of vitamins A and E on common biochemical analytes

In the multiple linear regression model, total bilirubin, direct bilirubin, alanine aminotransferase, glutamyl transpeptidase, alkaline phosphatase, lactate dehydrogenase, urea, glucose, uric acid, total

TABLE 2 Sex-specific reference intervals for vitamins A and E

	Total				Females				Males				p
	LL	90% CI	UL	90% CI	LL	90% CI	UL	90% CI	LL	90% CI	UL	90% CI	
Vitamin A	0.283	0.271–0.297	0.730	0.708–0.752	0.280	0.261–0.300	0.700	0.671–0.729	0.286	0.269–0.304	0.753	0.720–0.786	0.257
Vitamin E	4.39	4.15–4.64	15.63	14.95–16.34	4.51	4.01–5.04	16.14	15.29–17.00	4.41	4.16–4.68	14.67	13.72–15.69	<0.05
Vitamin E/TC	2.61	2.52–2.71	7.68	7.30–8.10	2.65	2.48–2.83	7.71	7.23–8.23	2.60	2.48–2.72	7.60	7.04–8.23	0.016

Note: Values of Vitamin A and E are in milligrams per liter; TC, total cholesterol. The units for Vitamin E/TC is umol/mmol.

Abbreviations: CI: confidence interval; LL, lower limit; UL, upper limit.

cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels showed statistical significance ($p < 0.05$). After adjustment for sex and age, the alanine aminotransferase, glutamyl transpeptidase, urea, glucose, and uric acid levels increased with increasing vitamin A levels ($p < 0.05$), while the total cholesterol and low-density lipoprotein cholesterol levels increased with increasing vitamin E levels ($p < 0.05$). However, direct bilirubin levels decreased with increasing vitamin E levels ($p < 0.05$) (Table 3).

4 | DISCUSSION

Vitamins A and E, which are fat-soluble chemicals, play a crucial role in sustaining life activities. Thus, evaluating vitamin A and E status requires accurate quantification. The gold standard for measuring vitamin D levels is LC-MS/MS, which has high specificity and sensitivity and prevents interference from nonspecific reactions and cross-reactivity, leading to greater accuracy and reliability. In our laboratory, we developed an accurate and reliable LC-MS/MS method for establishing vitamin A and E RIs that can be used in the clinical setting. Furthermore, a recent study revealed a stable positive interrelation between plasma concentrations of vitamins E along the trajectory of ageing independent of the other identified determinants.[16] Therefore, it is essential for clinical laboratories to establish RIs based on their current measurement methods, especially when these values are applied to the elderly population. In this study, vitamin A and E levels in fasting serum samples were measured to reflect the individual's reserve status.

We observed significantly higher serum vitamin E levels in females than in males in this study. However, in a study by Liu et al.,[8] Chinese elderly males showed adequate dietary vitamin E intake because of increased consumption of cooking oil, meat, and sugar. This finding could explain the slightly higher dietary vitamin E intakes in older men than in older women, which may be due to the differences in the level of lipids responsible for transporting fat-soluble vitamins between males and females. Additionally, the study by Liu et al. was conducted in 2010–2012, and the nutritional lifestyles of elderly women might have already changed by 2019. Meanwhile, a study by Kamisha [17] found no statistical sex-specific difference in vitamin E levels in children. Most studies on vitamin A and E RIs focused on children, pregnant women, or adults aged 18–65 years,[18-20] while only few focused on elderly individuals. In our study, however, we focused on the elderly and found no sex-specific differences in serum vitamin A levels in this population.

The Mayo Clinic Laboratories proposed RIs for serum vitamin A and E as 0.325–0.780 and 5.50–17.00 mg/L, respectively, for individuals aged >18 years, based on an LC-MS/MS method (https://www.mayocliniclabs.com). In our laboratory, we established slightly lower RIs of 0.283–0.730 and 4.39–15.63 mg/L for vitamins A and E, respectively, in elderly individuals. Using a high-performance liquid chromatography method, Johnson-Davis et al.[17] showed that the RIs for vitamins A and E were 0.260–0.609 and 4.80–12.77 mg/L, respectively, in children. These findings

TABLE 3 Effects of vitamins A and E on common biochemical analytes

Assay	Model test *p*-value	Vitamin A		Vitamin E		Sex		Age	
		Beta	*p*	Beta	*p*	Beta	*p*	Beta	*p*
TP	–	Excluded	–	Excluded	–	Excluded	–	Excluded	–
Alb	–	Excluded	–	Excluded	–	Excluded	–	Excluded	–
TBil	<0.001	Excluded	–	Excluded	–	0.266	<0.001	0.152	0.003
DBil	<0.001	Excluded	–	−0.111	0.04	0.181	0.001	Excluded	–
ALT	0.002	0.168	0.002	Excluded	–	Excluded	–	Excluded	–
GGT	<0.001	0.237	<0.001	Excluded	–	Excluded	–	Excluded	–
ALP	<0.001	Excluded	–	Excluded	–	−0.185	<0.001	Excluded	–
AST	–	Excluded	–	Excluded	–	Excluded	–	Excluded	–
LD	0.004	Excluded	–	Excluded	–	−0.115	0.015	0.131	0.032
Potassium	–	Excluded	–	Excluded	–	Excluded	–	Excluded	–
Sodium	–	Excluded	–	Excluded	–	Excluded	–	Excluded	–
Chloride	–	Excluded	–	Excluded	–	Excluded	–	Excluded	–
Urea	<0.001	0.227	<0.001	Excluded	–	Excluded	–	0.181	<0.001
Glu	0.019	0.125	0.019	Excluded	–	Excluded	–	Excluded	–
UA	<0.001	0.240	<0.001	Excluded	–	0.295	<0.001	0.109	0.027
TC	<0.001	Excluded	–	0.390	<0.001	Excluded	–	Excluded	–
TG	–	Excluded	–	Excluded	–	Excluded	–	Excluded	–
HDL-C	<0.001	Excluded	–	Excluded	–	−0.323	<0.001	Excluded	–
LDL-C	<0.001	Excluded	–	0.360	<0.001	Excluded	–	Excluded	–

Note: Beta, standardized beta coefficient; Excluded, the difference in levels of this factor was not statistically significant and could not be entered into the multifactorial model.

Abbreviations: Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DBil, direct bilirubin; GGT, glutamyl transpeptidase; Glu, glucose; HDL-C, high-density lipoprotein cholesterol; LD, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; TBil, total bilirubin; TC, total cholesterol; TG, triglycerides; TP, total protein; UA, uric acid. $p<0.05$ was considered statistically significant.

indicate that RIs for vitamins A and E vary among different populations. To our knowledge, this study is the first to establish the RIs for serum vitamin A and E levels in Chinese elderly people using an LC-MS/MS method.

This study has some limitations. Due to its retrospective design, we were unable to download data from the Laboratory Information System such as dietary habits and intake of health supplements. In addition, an age-partitioned analysis of the elderly was not conducted due to the limited sample size. Furthermore, it was not possible for us to determine whether vitamin A or E was responsible for the changes in biochemical analyte levels. However, we found correlations between vitamins A and E and biochemical analytes, and these findings could provide clues for future prospective studies.

In conclusion, this study established RIs for serum vitamins A and E in Chinese elderly people using an LC-MS/MS method. We also found that females had significantly higher vitamin E levels than males; however, no significant difference was found for vitamin A levels. Our findings may contribute to the assessment of micronutrient status in the elderly. In the future, we will establish the senescence model related to nutrition elements based on big data, providing a reliable basis for the study of biological effects and senescence.

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Qiu Ling designed the research. Yin Yicong and Ma Chaochao wrote the manuscript. Wang Danchen collected the specimens.Yin Yichong, Yu Songlin, Shaowei Xie and Qian Cheng carried out specimen testing. Ma Chaochao analyzed the data.

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CONFLICT OF INTEREST

The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the report for publication.

AUTHOR CONTRIBUTIONS

All authors have accepted responsibility for the entire content of this submitted manuscript and approved its submission.

EMPLOYMENT OR LEADERSHIP

None declared.

HONORARIUM

None declared.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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