Biol Trace Elem Res (2015) 165:18–29
DOI 10.1007/s12011-015-0233-7

A Correlation Between Diet and Longevity Characterization by Means of Element Profiles in Healthy People over 80 Years from a Chinese Longevous Region

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Received: 15 October 2014 / Accepted: 11 January 2015 / Published online: 21 January 2015

Abstract Monitoring the element concentrations in the human body is of critical importance for health and longevity. In order to explore the formation mechanism of longevity from the perspective of the body's element loads, this study investigated the prominent feature of element profiles in healthy people over 80 years from Bama County (China), a famous longevous region (LR). The element profiles in nails of elderly people from the LR and a non-longevous region were determined by inductively coupled plasma mass spectrometry, using orthogonal projections to latent structures discriminant analysis (OPLS-DA) for pattern recognition. As a result, four characteristic elements closely related to the healthy elderly people from LR, including Cr, Fe, Mn, and Co, were identified. The concentrations of Cr, Fe, Mn, and Co were significantly increased in the LR group ($p<0.05$). The values of fold change of Cr, Fe, Mn, and Co were 3.00, 2.46, 2.24, and 2.21, respectively. These characteristic elements could provide an important material guarantee for health and longevity of elderly people in the LR. The further correlation analysis revealed significant positive correlations between the Sr ($r=0.886$), Mn ($r=0.873$), Ni ($r=0.786$), and Co ($r=0.738$) concentrations in nails of elderly people and those in drinking water. Furthermore, significant positive correlations were found between the Se ($r=0.940$), Mn ($r=0.833$), and Fe ($r=0.733$) concentrations in nails and their dietary intake. Consequently, the observations suggested that diet could provide extraordinary reference information in terms of reflecting the feature of element profiles in healthy elderly people over 80 years from the LR.

Keywords Longevous region · Elderly people · Nail · Element · Pattern recognition · Diet

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Introduction

The body's element loads are known to exert important influence on metabolism and aging process [1–3]. Some essential trace elements play an important role in sustaining healthy aging, which is evidenced by some epidemiological investigations. Essential element deficiencies may contribute to the initiation and progression of a number of chronic diseases [4], including age-related disorders, such as Alzheimer's disease [5]. Meanwhile, excessive accumulation of toxic elements in human tissues that results from heavy metal pollution may also pose health hazards for the human body [6]. Therefore, monitoring the amounts of trace elements in human body is of critical importance for health and longevity. Analyses of serum, urine, nail, or hair samples are commonly used to assess element levels in the body [7].

Nails are biological specimens that have some advantages as bio-indicator for the metal analysis, because the sample collection is simple and noninvasive, and the samples are easy to store and very stable during storage [8]. Furthermore, nails can reflect metabolic changes of many elements over long periods of time ranging between 12 and 18 months [9]. Therefore, nails are attractive biopsy materials to assess element levels in the human body [8].

Up to now, there are many studies regarding the assessment of in vivo contents of elements by means of analyzing element concentrations in nails. For example, a study discovered higher contents of As, Mn, Pb, and Ni in nails, hair, and skin scales of victims from an arsenic affected area [10]. Furthermore, it was reported that lower levels of Se and Zn in nails were associated with an increased risk of children asthma [11]. Besides, another study demonstrated that low levels of Se and Zn and high levels of Cu, Fe, and Mn in nails and hair appeared to be related to high risk of prostate cancer [12]. In fact, most of these studies focused on the relationship between a specific disease or heavy metal pollution and in vivo contents of elements. Whereas, a study on applying computer pattern recognition technology to investigate the feature of element profiles in healthy elderly people over 80 years has not been reported yet.

In molecular biological level, there are both synergistic and antagonistic interactions between elements [13, 14]. Human health and longevity are related with overall element concentrations rather than individual ones, that is to say, the elemental homeostasis in the human body. As multivariate statistical techniques take the overall element concentrations and the interactions between elements into account, the use of pattern recognition technology combined with multivariate statistical methods is necessary to investigate the feature of element levels in the human body.

As a mature pattern recognition technique, orthogonal projections to latent structures discriminant analysis (OPLS-DA) is also a multivariate statistical method. It can take the overall elements as well as the interaction and coaction between elements into account to explore the feature of element profiles. It has superiority in solving the classification problem with a very small number of samples and high dimensional pattern recognition problems [15]. Dimension reduction is a distinct advantage of OPLS-DA. Furthermore, OPLS-DA can distinguish ‘among-groups’ and ‘within-groups’ variability, and thereby extract the most relevant information in differences among groups. The classification error rate of OPLS-DA is much lower than traditional discriminant analysis methods. Relevant studies have shown that OPLS-DA is also much more robust for small sample size [16]. In consideration of the limited number of samples, we used this method in the present study to build the classification model and identify characteristic elements. To date, the studies on applying OPLS-DA for pattern recognition only focus on the relationship between a specific disease and element profiles in human body [17, 18]. However, there are no reports on using OPLS-DA to investigate the feature of element profiles in nails of healthy elderly people.

The element concentrations in nails are inevitably affected by the element levels in diet. Oyoo-Okoth E et al. reported the relationships between element concentrations in nails of children with those in 10 specific food items along with water [19]. However, we have found no published data referring to relationships between the nail element concentrations and dietary mineral intake which was obtained by means of comprehensive assessment of overall dietary nutrition status. Whereas, given the important influence of overall diet on the nail element concentrations, it is essential to illustrate the correlations between the element contents in nails and dietary mineral intake.

There is an amazing phenomenon in Bama County, Guangxi Province, China. The number of centenarians was 80 in the population of 224,637 [20]. It means that the ratio of centenarians per 100,000 inhabitants has reached up to 36.5 in Bama County in 2010. To date, to our knowledge, the ratio of centenarians in Bama County should be the highest in China. The phenomenon of improved human health and increased longevity is prominent in Bama County, bearing distinctive local features. It is reported that some trace elements play important roles in maintaining the metabolic homeostasis in elderly people [21] and the prevention of many age-associated diseases, and maintaining the normal immune function [2, 22]. The appropriate element levels in the body may be beneficial to health and longevity. Therefore, element profiles in nails and element metabolism in the body of healthy elderly people from the Bama longevous region (LR) should have remarkable and anticipated features. However, few studies have been focused on the element profiles in nails of elderly people living in the LR due to their traditional and conservative lifestyle. Therefore, current data and information could be a valuable reference.

Based on these facts, in this study, we analyzed the element concentrations in nails of healthy elderly people from the LR. We further explored the feature of element profiles in nails of elderly people from the LR by means of OPLS-DA. Furthermore, the present study assessed the correlations between nail element concentrations and dietary intake of elements, as well as those element concentrations in drinking water. This will provide a reference for coming research on element metabolism in healthy elderly people, and serve coming nutritional and health studies, which could help in elucidating the complex formation mechanism of longevity from the perspective of the body’s element loads.

Materials and Methods

Sample Collection and Preparation

The LR group consisted of 10 healthy elderly people aged over 80 years from the LR. The control group was composed of eight healthy elderly people aged over 80 years from a non-longevous region, Xixiangtang District in Guangxi Province, where the ratio of centenarians was the lowest in Guangxi Province and the geographical environment and climatic environment were similar with Bama County. To guarantee that each study sample was of a relatively healthy elderly person, the slightly modified criteria based on the criteria of Darviri C were adopted [23]. The admission criteria included that (a) the subjects should be volunteers and grant an interview, (b) be able to adequately communicate with the interviewer, (c) not be bedridden, (d) be relatively functional (i.e., be able to move in and out of their houses and be self-served), and (e) be natives. Furthermore, according to the data of routine health examination of elderly people over 80 years conducted in the local hospitals, subjects suffering from chronic or acute diseases were excluded. As a matter of fact, there were very few centenarians who met the admission criteria in the non-longevous region. Therefore, to ensure that the subjects in the control group were age-matched with the subjects in the LR group, healthy elderly people over 80 years who participated routine health examinations and met the above admission criteria were selected as the subjects of the study, which had tremendous potential in healthy and successful aging.

The personal details such as age, sex, residential address, occupation, education, and hobbies were recorded on a regular pro forma at the time of sample collection from the subjects. Their height and weight were measured and recorded. The LR group consisted of five males and five females, with a mean age of 87.50±8.05 years old. The control group consisted of four males and four females, with a mean age of 86.63± 4.90 years old. There is no significant difference in age between the two groups ($p>0.05$). The subjects were matched according to age and sex, excluding the influences of age factor and sex factor on element levels in human body.

Nails were collected from 18 subjects with stainless steel nail cutters and stored in clean separate bags between January and October 2013. Tap water samples were collected from the subjects in January, April, July, and October 2013. The water samples were acidified with 1.8 % (*w*/*w*) nitric acid as acidification minimizes the adsorption of metals into the walls of the container and then stored at approximately 4 °C [6].

Dietary data were collected by four season consecutive 7-day weighed dietary records (28-day WDRs) [24, 25], which were carried out in January, April, July, and October 2013. During the dietary survey, the subjects were required to adopt the mode of individual dining and advised to maintain usual eating habits. With the cooperation of the subject and his offspring, the trained investigators weighed and recorded all food and drinks consumed by the subject using electronic food scales, measuring cups, and spoons. For unmeasurable food, standard portion size was applied. The trained dietitians checked the records on dietary survey site.

Instrumentation

Nail samples were decomposed with CEM Mars automatic microwave digester. All samples were analyzed by Agilent 7700e inductively coupled plasma mass spectrometry with a collision/reaction cell (CRC-ICP-MS).

Reagents

All the reagents were of guaranteed-reagent grade or better. High-purity deionized water (resistivity 18.2 MΩ cm) obtained from a Milli-Q water purification system (Millipore Inc., Billerica, MA, USA) was used throughout. All implements that came into contact with the samples were cleaned by overnight soaking in 47.6 % (*w*/*w*) HNO_3, followed by repeated rinsing with Milli-Q water. Stock single-element standard solutions (1000 μg/mL) of Na, Mg, K, Ca, Mn, Fe, Cu, Zn, As, Sn, Sb, Pb, Cr, Co, Ni, Se, Sr, and Ba were purchased from National Standard Materials Study Center, China. Internal standard mix (10 μg/mL) which contains scandium (Sc), germanium (Ge), indium (In), and bismuth (Bi) was purchased from Agilent Technologies (Santa Clara, CA, USA).

Element Analysis

Before washing the nail samples, any visible dirt on the surface of nails was scraped with a clean stainless steel scalpel to remove external filth [26]. Then the nails were thoroughly washed using an ultrasonic bath with Milli-Q water for 30 min followed by washing again with acetone for 30 min in an ultrasonic bath. Finally, the nails were washed with Milli-Q water for 30 min in an ultrasonic bath. The washed samples were dried at 50 °C overnight in a drying oven [10].

Approximately, 0.1 g of dried nails from each subject was weighted into a dry, clean digestion vessel [10]. Then 5 mL of nitric acid (65 %) and 1 mL of hydrogen peroxide (30 %) were added. The mixture including nails, nitric acid, and hydrogen peroxide was treated with microwave. The microwave digestion was carried out using the following program: (i) 5 min at 1600-W power and 120 °C (ramp 5 min), (ii) 5 min at 1600-W power and 150 °C (ramp 5 min), and (iii) 20 min at 1600-W power and 180 °C (ramp 7 min). When digestion was complete, the vessels were cooled to room temperature. At last, the mixtures were diluted to 50 mL with Milli-Q water and used for element determination. A blank sample was prepared by exactly the same procedure [10].

Concentrations of Na, Mg, K, Ca, Mn, Fe, Cu, Zn, As, Sn, Sb, Pb, Cr, Co, Ni, Se, Sr, and Ba in nails and drinking water were determined by ICP-MS under optimized conditions. The instrumental conditions were set as follows: radio-frequency power 1550 W, plasma gas flow rate 15 L/min, auxiliary gas flow rate 1.0 L/min, carrier gas flow rate 1.0 L/min, sample depth 8 mm, spray chamber temperature 2 °C, and data acquisition repetition three. For avoiding polyatomic interferences, the instrument was operated in the octopole collision system mode. Collision cell was pressured with helium at a flow rate of 4.5 mL/min. The octopole and quadrupole voltages were adjusted to −18 and −15 V, resulting in a kinetic energy discrimination of 3 V. The internal standards containing ^{45}Sc, ^{72}Ge, ^{115}In, and ^{209}Bi were on-line merged into the sample flow to negate the possible mixing problems [27]. For the quantification, standard external calibrations curves were generated from standard solutions. According to the element concentrations in most of the nail samples, the calibration range of each element was determined. Analytical calibration standards were prepared over the range of 0–500 (Na), 0–200 (Mg), 0–100 (K), 0–2500 (Ca), 0–10 (Mn), 0–100 (Fe), 0–20 (Cu), 0–500 (Zn), 0–0.8 (As), 0–0.3 (Sn), 0–0.5 (Sb), 0–1.5 (Pb), 0–2 (Cr), 0–0.2 (Co), 0–3 (Ni), 0–2 (Se), 0–2 (Sr), and 0–2.5 μg/L (Ba), respectively, by suitable serial dilutions of each stock solution in 4.6 % (*w*/*w*) HNO_3. Linearity measured as the correlation coefficient was higher than 0.999 for all elements.

To check for instrumental drift, one of these multielement standard solutions with known metal concentrations was analyzed for every 10 samples. Due to the unavailability of appropriate commercial-certified nail reference material for the validation of our method, a standard addition method was performed with several nail samples, which were spiked with standards and subjected to the same digestion and analysis procedures. Satisfactory recovery rates of 80–109 % were obtained for all elements and procedural replication showed RSD <10 % for all analyzed elements (n=5). These result validated that the method was accurate and precise. Therefore, the method is adoptable to apply in the study.

Statistical Analysis

The nail element data derived from ICP-MS was imported into the SIMCA-P 11.5 software package (Umetrics, Umeå, Sweden) for multivariate statistical analysis. To alleviate the influence of the heteroscedasticity originating from the concentration variations of different elements on the multivariate statistical model, the data was mean-centered and scaled to unit variance (UV) prior to principal component analysis (PCA) and OPLS-DA [28, 29]. Unsupervised PCA was performed to visualize intrinsic variations of element levels in elderly people from the two regions at a global fashion. A more sophisticated discriminant technique OPLS-DA that is suitable for very small sample sizes was further applied to achieve global separation profile between inter-groups. The loading of OPLS-DA model was then combined with the value of the variable importance in the projection (VIP) calculated from the model to identify the differential elements contributing to the variations between the two groups [30, 31]. Consequently, the characteristic elements that were closely related to the elderly people over 80 years from the LR were determined. For OPLS-DA modeling in SIMCA-P software, a typical sevenfold (Leave–1/7th Samples–Out) cross-validation procedure was carried out to avoid model overfitting. Furthermore, a nonparametric Kruskal-Wallis test in SPSS 17.0 software was used to determine if a significant difference of each differential element from multivariate statistical analysis existed between the two groups. The critical p value was set as 0.05 in this study. Moreover, fold change (FC) was calculated by the ratio of mean rankings of the element concentrations in the longevous region group versus the control group.

Spearman correlation test in SPSS 17.0 software was used to evaluate correlations between element concentrations in nails and drinking water. The statistical significance was set at $p<0.01$ and $p<0.05$, respectively.

The data on weighed dietary records was converted to dietary mineral intake according to the Chinese food composition tables [32]. Average daily mineral intakes were computed by multiplying the quantities of food consumed (in grams) or portion size by the mineral contents per 100 g of food listed in the Chinese food composition tables. Spearman correlation test was used to evaluate correlations between element concentrations in nails and dietary mineral intake. The statistical significance was set at $p<0.01$ and $p<0.05$, respectively.

Results

Pattern Recognition Analysis of Element Profiles in Nails

To analyze the chemical element contents in nails of the healthy elderly people in the LR group and control group, we performed using ICP-MS. The results are presented in Table 1. Ca was the highest concentration in nails, with average levels of 664.8±233.0 μg/g dry nail weight in the LR group and 525.6±346.0 μg/g in the control group respectively. Furthermore, some elements were substantially spread around the mean concentration levels, indicated by the large standard deviation values, which suggested that the samples of the study were extremely heterogeneous. In a small number of nail samples, the contents of Sr, Co, Cr, Pb, Sb, Mn, and K were below their corresponding limit of quantification (LOQs). When the element levels were under the respective LOQs, concentrations were assumed to be a half of the corresponding LOQs.

Table 1 Basic statistical parameters for chemical elements in nails of elderly people

Element	Group	Mean (μg/g)	Standard deviation (μg/g)	Median (μg/g)	Maximum (μg/g)	Minimum (μg/g)	LOQ (μg/g)	p
Na	LR	117.035	134.120	80.064	365.589	7.050	1.280	0.633
	Control	166.612	162.567	122.553	422.637	2.090		
Mg	LR	52.919	11.962	54.037	74.221	34.856	0.397	0.573
	Control	52.220	27.686	58.860	86.355	1.620		
K	LR	1.593	1.724	1.132	5.455	<LOQ	0.979	0.360
	Control	37.313	91.130	3.529	262.149	<LOQ		
Ca	LR	664.804	232.954	680.772	1071.952	241.675	4.878	0.515
	Control	525.573	345.993	586.054	961.091	10.126		
Mn	LR	1.363	0.815	1.094	3.063	0.501	0.054	0.004*
	Control	0.346	0.477	0.189	1.361	<LOQ		
Fe	LR	27.589	9.154	24.531	42.182	17.093	0.093	0.001*
	Control	10.734	7.111	9.097	26.319	3.684		
Cu	LR	5.066	1.929	4.643	9.279	3.089	0.019	0.055
	Control	3.244	1.651	3.406	5.431	0.106		
Zn	LR	129.907	24.895	123.158	182.706	102.763	0.024	0.203
	Control	115.402	18.166	112.996	147.825	96.229		
As	LR	0.195	0.054	0.195	0.273	0.098	0.005	0.274
	Control	0.157	0.090	0.122	0.308	0.063		
Sn	LR	0.048	0.028	0.044	0.099	0.018	0.005	0.745
	Control	0.058	0.036	0.049	0.143	0.030		
Sb	LR	0.074	0.194	0.012	0.624	<LOQ	0.003	0.050*
	Control	0.005	0.005	0.005	0.012	<LOQ		
Pb	LR	0.251	0.156	0.242	0.520	<LOQ	0.004	0.984
	Control	0.243	0.188	0.229	0.520	<LOQ		
Cr	LR	0.594	0.200	0.556	0.979	0.386	0.007	0.000*
	Control	0.026	0.042	<LOQ	0.097	<LOQ		
Co	LR	0.022	0.014	0.018	0.054	0.006	0.003	0.005*
	Control	0.006	0.015	<LOQ	0.042	<LOQ		
Ni	LR	0.713	0.358	0.558	1.274	0.321	0.065	0.714
	Control	0.631	0.271	0.655	1.131	0.299		
Se	LR	0.156	0.241	0.046	0.646	0.033	0.019	0.814
	Control	0.044	0.008	0.041	0.059	0.038		
Sr	LR	0.321	0.188	0.285	0.655	<LOQ	0.005	0.761
	Control	0.290	0.303	0.270	0.749	<LOQ		
Ba	LR	0.605	0.328	0.481	1.229	0.310	0.007	0.173
	Control	0.408	0.287	0.353	0.905	0.010		

Data about the elements are expressed in micrograms per gram. The sample size for the longevous region group and control group is 10 and 8, respectively. A LOQ was determined by 10 σ criteria using the standard deviation σ of 10 blank measurements. The *p* values are obtained by Kruskal-Wallis test

LR longevous region, *LOQ* limit of quantification.

*$p \leq 0.05$, significant difference

To evaluate the statistical differences between the LR group and control group, data were analyzed by nonparametric Kruskal-Wallis test. The results are shown in Table 1. The contents of Mn (p=0.004), Fe (p=0.001), Sb (p=0.050), Cr (p=0.000), and Co (p=0.005) were significantly higher for the LR group, as compared to the control group.

The concentrations of Mn, Fe, Cu, Zn, Pb, Cr, Co, and Ni in nails were in the same range as in other population living in different areas including Henan Province and Hubei Province in Central China, while the Ba, Sr, and Se levels were comparatively lower than those in their results [5]. This could be attributed to the geological and geographical differences in the two distinct study areas and to the difference of age of the subjects.

Multivariate statistical analysis, including PCA and OPLS-DA, was performed to build the classification model and identify differential elements between groups. The PCA scores plot depicted a clear separation trend between the LR group and control group. Furthermore, the two groups exhibited complete and significant separation in the scores plot of OPLS-DA model that can maximize systematic variations between the two groups, as shown in Fig. 1. This indicated that

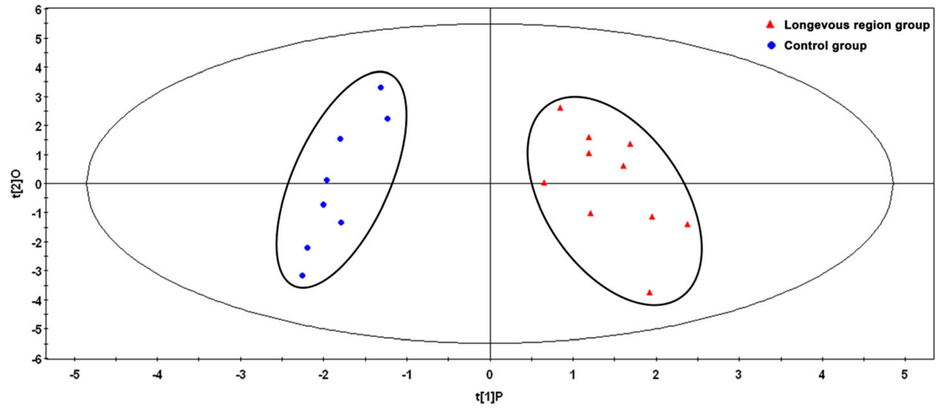

Fig. 1 Scores plot of OPLS-DA model of ICP-MS data obtained from the longevous region group (*red triangles*) and control group (*blue dots*). Each dot represents an individual subject and the spatial distribution of these dots reveals the variations of element profiles in nails of healthy elderly people in the longevous region group and control group. The two groups exhibit complete and significant separation in the scores plot of OPLS-DA model, indicating that the longevous region group and control group have significant differences in the element profiles in nails

the LR group and control group had distinct differences in the element profiles in nails, and the intergroup differences were more significant although there was some intrinsic biological variability of each person. The results suggested that there were distinctive features of element metabolism between the human body in the LR group and control group.

The modeling quality is extremely important for OPLS-DA. In order to assess OPLS-DA modeling quality, the parameters of OPLS-DA model are summarized in Table 2. R^2X(cum) and R^2Y(cum) represent the cumulative modeled variation in X and Y matrices, respectively, by means of the two parameters to assess the goodness of fit of the OPLS-DA model. Q^2(cum) is the cumulative predicted variation. The values of these parameters close to 1.0 indicate a reliable mathematical model with satisfactory predictability, while OPLS-DA models with Q^2(cum) value exceeding 0.4 are acceptable in practical applications. As shown in Table 2, the OPLS-DA model (R^2X(cum)=0.557, R^2Y(cum)=0.931, Q^2(cum)=0.758) demonstrated satisfactory modeling and predictive abilities using one predictive component and two orthogonal components.

To identify which elements were accountable for such significant separation, variable importance in the projection (VIP) statistics was initially used to select the differential elements between the two groups. The VIP plot with jack-knifed based confidence interval ($CIJF_{jk}$) of OPLS-DA model is shown in Fig. 2. The VIP statistics ranked the overall contribution of each element to the OPLS-DA model, and those elements with VIP >1.0 were considered statistically significant in this model. Moreover, $CIJF_{jk}$ was used for further choosing the differential elements between the two groups, which displayed the uncertainty of each element and the smaller span of confidence interval the higher creditability of the selected element. In this study, those elements with $CIJF_{jk}$ (α=0.05) across zero were excluded. Therefore, our study only selected those elements meeting the two criteria, that is to say, VIP >1.0 and the span of $CIJF_{jk}$ excluding zero, as the differential elements identified by multivariate statistical analysis, including Cr, Fe, Mn, Co, and Cu. The VIP values of the five elements are presented in Table 3. It indicated that Cr, Fe, Mn, Co, and Cu could serve as the significant and reliable differential elements contributing to the variations between the LR group and control group.

The differential elements were further validated by the loadings plot of OPLS-DA model, as shown in Fig. 3. In the loadings plot, each triangle denoted an individual element. The triangles far away from the origin represented the elements responsible for the differences between the two groups. The abovementioned differential elements, including Cr, Fe, Mn, Co, and Cu, were the farthest away from the origin in the loadings plot, which further indicated that Cr, Fe, Mn, Co, and Cu were the differential elements obtained from the OPLS-DA model. Moreover, the concentrations of these elements in nails in the LR group were significantly higher than those in the control group, which could be regarded as potential characteristic elements related to the elderly people aged over 80 years from the LR.

To verify the statistically significant difference of the differential elements derived from multivariate statistical analysis, the abovementioned potential characteristic elements identified by the OPLS-DA model were further validated at

Table 2 Summary of the parameters for assessing OPLS-DA modeling quality

	Component	R^2X(cum)	R^2Y(cum)	Q^2(cum)
Longevous region group vs. control group	1P+2O	0.557	0.931	0.758

R^2X(cum) and R^2Y(cum) represent the cumulative modeled variation in X and Y matrices, respectively, and Q^2(cum) is the cumulative predicted variation. 1P+2O, one predictive component and two orthogonal components for establishing the OPLS-DA model

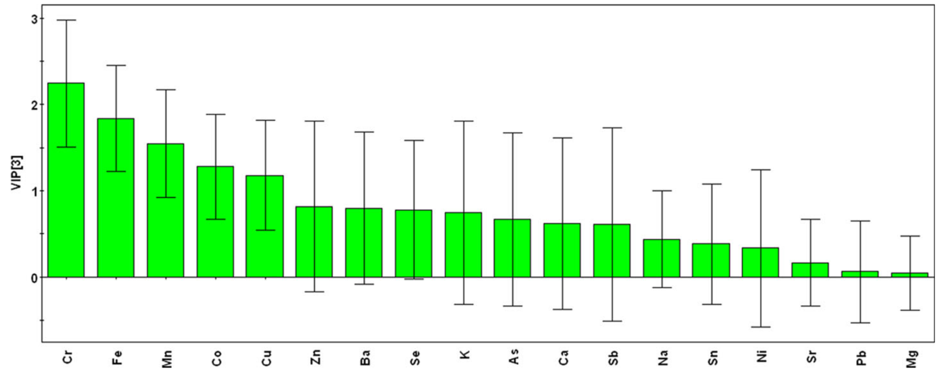

Fig. 2 VIP plot with $CIJF_{jk}$ of OPLS-DA model of ICP-MS data. The elements meeting the two criteria, that is to say, VIP >1.0 and the span of $CIJF_{jk}$ excluding zero, are selected as the differential elements identified by multivariate statistical analysis, including Cr, Fe, Mn, Co, and Cu, which can serve as the significant and reliable differential elements contributing to the variations between the longevous region group and control group

a univariate level. According to the results of Kruskal-Wallis test, Cr, Fe, Mn, and Co were significant differential elements between the two groups ($p<0.05$). Furthermore, the concentrations of Cr, Fe, Mn, and Co were significantly increased in the LR group. The values of fold change (FC) of Cr, Fe, Mn, and Co were 3.00, 2.46, 2.24, and 2.21, respectively. Therefore, the significant differential elements between the LR group and control group, including Cr, Fe, Mn, and Co, were finally obtained by multivariate statistical methods coupled with a univariate statistical method such as Kruskal-Wallis test. These elements could serve as characteristic elements related to the elderly people over 80 years from the LR.

Correlations Between Element Concentrations in Nails and Drinking Water

In order to assess the correlations between element concentrations in nails and drinking water, the element contents in drinking water of the healthy elderly people over 80 years from the LR were analyzed by ICP-MS. The results are presented in Table 4, and the data about the elements are expressed in micrograms per liter. Ca (49,606.875±38,226.387 μg/L) was the highest concentration in drinking water, followed by Na (2386.750±1702.757 μg/L), Mg (2017.250±943.492 μg/L), Zn (1724.648±1320.381 μg/L). The concentrations of some microelements in drinking water, such as Co (0.032±0.040 μg/L), Se (0.082±0.052 μg/L), Sn (0.084±0.091 μg/L), Sb (0.153±0.085 μg/L), Cr (0.378±0.269 μg/L), As (0.399±0.771 μg/L), Ni (2.213±2.299 μg/L), and Pb (2.472±2.180 μg/L), were relatively lower. The relevant research has shown that drinking water with appropriate mineral element concentrations is conducive to human health, and the imbalance of elements in drinking water can lead to some diseases [33]. Therefore, it is implied that the element profiles in drinking water in this LR, to some extent, may have positive influence on local longevity.

With Spearman rank correlation test, this study found a number of significant correlations between the elements in nails and the corresponding elements in drinking water of the elderly people from the LR, as shown in Table 5. Significant positive correlations were observed between the Sr (r=0.886), Mn (r=0.873), Ni (r=0.786), and Co (r=0.738) concentrations in nails and drinking water. This indicated that drinking water was an important source of Sr, Mn, Ni, and Co in elderly people aged over 80 years from the LR.

Table 3 Differential elements derived from OPLS-DA model with Kruskal-Wallis test

Element	VIP value	Fold change	p value (Kruskal-Wallis test)
Cr	2.242	3.00	0.000
Fe	1.837	2.46	0.001
Mn	1.547	2.24	0.004
Co	1.281	2.21	0.005
Cu	1.179	1.73	0.055

Variable importance in the projection (VIP) is obtained from OPLS-DA with a threshold of 1.0. Fold change (FC) is calculated by the ratio of mean rankings of the element concentrations in the longevous region group versus control group using Kruskal-Wallis test and the p values are obtained accordingly. The critical p value is set as 0.05

Correlations Between Element Concentrations in Nails and Dietary Mineral Intake

The daily mineral intakes of the elderly people over 80 years from the LR were obtained by four season consecutive 7-day WDR method coupled with the Chinese food composition tables. Na (1950.67±397.88 mg/day) was the highest intake, followed by K (1492.00±337.87 mg/day), Ca (497.33±117.92 mg/day), Mg (353.97±67.84 mg/day), Fe (14.17±3.60 mg/day), Zn (6.49±2.73 mg/day), Mn (3.15±1.13 mg/day), Cu (1.16±0.28 mg/day), and Se (23.23±16.94 μg/day).

Table 6 shows the correlations between the element concentrations in nails and the corresponding mineral intake of

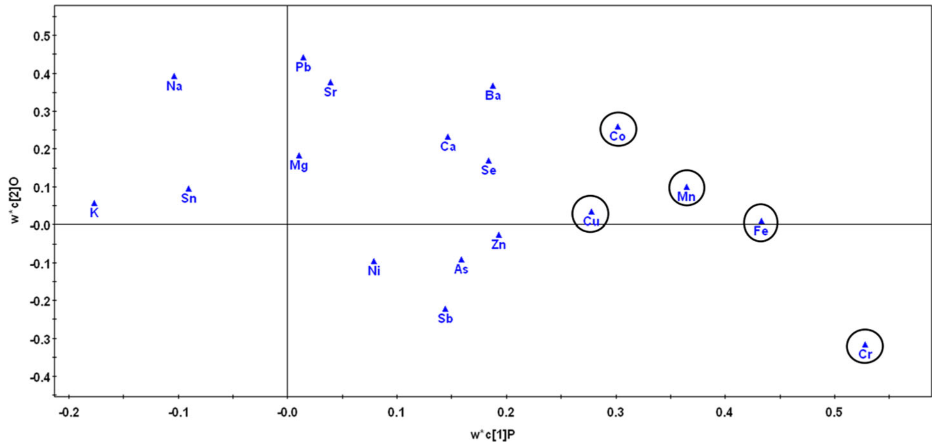

Fig. 3 Loadings plot of OPLS-DA model Each *triangle* denotes an individual element. The *triangles* far away from the origin represent the elements responsible for the differences between the two groups. Cr, Fe, Mn, Co, and Cu are the farthest away from the origin in the loadings plot, which further indicates that Cr, Fe, Mn, Co, and Cu are the differential elements obtained from the OPLS-DA model

the elderly people from the LR. Significant positive correlations were found between the Se (r=0.940), Mn (r=0.833), and Fe (r=0.733) concentrations in nails and their dietary intake. This indicated that the daily diet was an important source of Se, Mn, and Fe in elderly people aged over 80 years from the LR.

Discussion

The most striking feature of this work is that we captured the characteristics of element profiles in nails of elderly people over 80 years from the LR by means of pattern recognition analysis coupled with a univariate statistical test. The results of pattern recognition showed that the nail element profiles of LR group were distinctively separated from those of control group by means of OPLS-DA model. Four characteristic elements closely related to the healthy elderly people from the LR, including Cr, Fe, Mn, and Co, were identified by OPLS-DA model combined with Kruskal-Wallis test. The present study is the first to discover that the concentrations of Cr, Fe, Mn, and Co were significantly increased in the LR group (p<0.05). The values of FC of Cr, Fe, Mn, and Co were 3.00, 2.46, 2.24, and 2.21, respectively. In addition, the results suggested that OPLS-DA could be used to manifest the different patterns of element profiles in nails of different populations.

Table 4 Element concentrations in drinking water of elderly people from LR

Element	Mean±standard deviation (μg/L)	Element	Mean±standard deviation (μg/L)
Na	2386.750±1702.757	Sn	0.084±0.091
Mg	2017.250±943.492	Sb	0.153±0.085
K	445.750±133.790	Pb	2.472±2.180
Ca	49,606.875±38,226.387	Cr	0.378±0.269
Mn	12.115±32.062	Co	0.032±0.040
Fe	82.324±40.566	Ni	2.213±2.299
Cu	14.987±21.370	Se	0.082±0.052
Zn	1724.648±1320.381	Sr	45.481±16.453
As	0.399±0.771	Ba	6.849±4.643

Data about the elements are expressed in micrograms per liter

These characteristic elements, including Mn, Fe, Co, and Cr, are essential elements for proper body physiology. They play an important role in physiological functions [34, 35]. Particularly, the key biochemical roles of Mn and Fe have been well known, due to their essential functions in antioxidant enzymes [36–41].

Mn is an activator or a cofactor of several enzymes [35]. In particular, Mn is an essential cofactor of mitochondrial superoxide dismutase (SOD) which is an important antioxidant enzyme [36, 38]. Mn-SOD plays an important role in cleaning up the free oxygen radicals [37, 40, 41]. Given that aging and most age-related diseases have their origin in deleterious free radical reactions, efficient removal of these free radicals slows the aging process [42, 43]. In addition, Mn plays an essential role in prevention of arteriosclerosis and cardiovascular disease [35]. Therefore, Mn is known as an anti-aging element [33]. Lv J et al. reported that the higher concentration of Mn in the hair of centenarians was conducive to their long lifespan [44].

Fe is a constituent of hemoglobin and myoglobin and plays an important role in oxygen delivery [34]. Moreover, Fe is present in antioxidant enzymes, for example, catalase and peroxidases [45]. Besides, Fe is a component of ferritin that plays a role as a protectant against oxygen free radical-mediated damage [39]. Thus, Fe is also a crucial element in the antioxidant defense system. In addition, Fe plays an important role in maintenance of normal immune function [46]. Lv J et al. reported that Fe content in rice had a weak positive effect on local longevity [33].

Co, a component of the vitamin B_{12} complex, is essential for the synthesis of red blood cells [36]. Besides, Co plays a biologically essential role as a cofactor in a number of proteins [47, 48]. Li Y et al. reported that Co positively affected the life span of centenarians [5]. Moreover, Liu Y et al. discovered that Co concentration in soil had a positive correlation with longevity indexes [49].

Cr^{3+} is an essential nutrient that potentiates insulin action and thus influences carbohydrate, lipid, and protein metabolism [35]. Besides, Cr^{3+} plays an important role in prevention of diabetes mellitus and cardiovascular disease [50]. Furthermore, Cr^{3+} is believed to have different kinds of humoral and cellular immune modulatory effects [50]. Moreover, Cr^{3+} deficiency may cause diminished longevity [51]. On the other side, Cr^{6+} is toxic and carcinogenic, which is mostly of industrial origin [50]. However, Cr^{3+} is the most common form in organisms, and almost all naturally found Cr is Cr^{3+} [50]. Given the living environment and chromium metabolism in the human body [50], it is almost impossible that Cr^{6+} is present in the elderly people from the LR. In the present study, Cr was analyzed as total Cr and no speciation analysis was carried out for Cr^{3+} or Cr^{6+}. Therefore, future studies on element speciation analysis are necessary to better explain our findings.

Consequently, from a point of view of the physiological functions of the trace elements, the results suggested that these characteristic elements provided an important material guarantee for health and longevity of the elderly people in Chinese LR. Taken all together, Mn, Fe, Co, and Cr should be particularly noteworthy in longevity characterization by means of element profiles in healthy elderly people over 80 years from the LR.

To date, the studies on investigating the feature of element profiles in nails, by means of OPLS-DA to conduct pattern recognition, are rather limited. There is currently no published information directly related to longevity characterization by means of element profiles in healthy elderly people, and hence no literature to directly support our results.

The elements cannot be synthesized by the human body and therefore must be obtained from food, drinking water, air, and through other routes. For most populations, the main route of exposure to metallic elements is through the diet (food and drinking water) [7]. Therefore, investigation of the relationship between the element concentrations in nails and drinking water as well as dietary mineral intake of elderly people from the LR would help to explore the underlying mechanism of longevity in the LR from point of view of element metabolism.

The present study assessed the correlations between nail element concentrations and dietary intake of elements, as well as those element concentrations in drinking water. The results showed that significant positive correlations were observed between the Sr (r=0.886), Mn (r=0.873), Ni (r=0.786), and Co (r=0.738) concentrations in nails and drinking water of the elderly people from the LR. On the other side, significant positive correlations were found between the Se (r=0.940), Mn (r=0.833), and Fe (r=0.733) concentrations in nails and their dietary intake of the elderly people from the LR.

Table 5 Correlations between element concentrations in nails and drinking water

Element	Na	Mg	K	Ca	Mn	Fe	Cu	Zn	As	Sn	Sb	Pb	Cr	Co	Ni	Se	Sr	Ba
r	0.548	0.643	−0.178	0.310	0.873**	0.119	0.190	0.595	−0.024	0.192	0.096	0.500	0.072	0.738*	0.786*	−0.108	0.886**	0.357
p	0.160	0.086	0.674	0.456	0.005	0.779	0.651	0.120	0.955	0.649	0.821	0.207	0.866	0.037	0.021	0.798	0.003	0.385

Spearman correlation test

r Spearman correlation coefficient

*p<0.05, correlations are statistically significant at the 0.05 level; **p<0.01, correlations are statistically significant at the 0.01 level

Table 6 Correlations between element concentrations in nails and dietary mineral intake

Element	Na	Mg	K	Ca	Mn	Fe	Cu	Zn	Se
r	0.050	0.192	0.410	−0.056	0.833**	0.733**	0.099	0.066	0.940**
p	0.858	0.494	0.129	0.844	0.000	0.002	0.725	0.815	0.000

Spearman correlation test

r Spearman correlation coefficient

**$p<0.01$, correlations are statistically significant at the 0.01 level

Biswas BK et al. reported a positive correlation between the arsenic concentrations in nails and drinking water of arsenic victims from an arsenic-affected area [52]. There were some differences between their results and the present results that showed a weak correlation between the arsenic concentrations in nails and drinking water. This could be attributed, firstly to the environmental differences in the two distinct study areas and secondly to the difference of age of the subjects.

Normally, dietary intake of elements is estimated based on the concentrations of elements in the consumed food items and the quantities of these food items consumed [53, 54]. However, accurately determining many element concentrations of total food items consumed is not realistic due to ingestion of a wide range of food items and the food items with different sources [19]. The relevant studies merely assessed intake of elements from the specific food items. No published data referring to the relationship between the element contents in nails and dietary mineral intake, by comprehensive assessment of overall dietary nutrition status, was found. Nevertheless, the present study was conducted to comprehensively assess the overall dietary nutrition status by quantitative 28-day WDR method coupled with the Chinese food composition tables, and thereby evaluate the correlations between the element concentrations in nails and dietary mineral intake. It may be a quantitative method of assessment when the element contents in various food items consumed is precisely known. Among available nutrition assessment approaches, the WDR method is the most precise and is accepted as a gold standard [55], although it is expensive, time-consuming, and requires considerable commitment on the part of the participants [56]. We carried out four season consecutive 7-day WDRs to assess habitual intake of minerals, minimizing variances in dietary intake according to seasons and days.

In this study, the stringent selection criteria led to the relatively small number of subjects. According to the data from the national bureau of statistics and the local hospitals, only these elderly people met our standards through screening in an all-round way. Therefore, these observations provided precious information which was of particular importance for coming trace element and longevity studies. Further studies including populations from different longevous regions are needed to confirm these findings.

Conclusion

The LR group and control group have significant differences in the element profiles in nails. Four characteristic elements closely related to the healthy elderly people from the LR, including Cr, Fe, Mn, and Co, were identified by OPLS-DA model combined with Kruskal-Wallis test. The concentrations of Cr, Fe, Mn, and Co were significantly increased in the LR group ($p<0.05$). The results suggested that these characteristic elements provided an important material guarantee for health and longevity of elderly people in the LR.

Moreover, significant positive correlations were observed between the Sr, Mn, Ni, and Co concentrations in nails and drinking water of the elderly people from the LR. On the other side, significant positive correlations were found between the Se, Mn, and Fe concentrations in nails and their dietary intake of the elderly people from the LR. Consequently, the abovementioned observations suggested that diet could provide extraordinary reference information in terms of reflecting the feature of element profiles in healthy elderly people over 80 years from the LR. In addition, the results showed us an interesting point of penetration to explore the formation mechanism of longevity.

Acknowledgments This study was supported by the grants from the National Natural Science Foundation of China (No. 31371762) and the Science Research and Technology Development Projects of Guangxi (No. 1222015-2A) and the “Bagui Scholars Distinguished Professor” Special Project. The authors are also deeply grateful to the 18 subjects and their offspring for their collaboration in 28-day WDRs and sample collection.

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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