Analysis of eight nutrient elements in peripheral blood of children and adolescents using inductively coupled plasma-mass spectrometry

Fang Luan
Shandong Provincial Hospital affiliated to Shandong First Medical University
Bin Liu
Shandong Provincial Hospital
Shuzhen Sun
Shandong Provincial Hospital
Yuan Chen
Shandong Provincial Hospital
Yanqiu Xu
Shandong Provincial Hospital
Xuerui Jiang
Shandong Provincial Hospital
Xiangrui Guo
Shandong Provincial Hospital
Shiqing Cheng
Shandong Provincial Hospital
Yong Wang (404389139@qq.com)
Shandong Provincial Hospital affiliated to Shandong First Medical University

Research

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Abstract

Background

Several researches have been conducted on the associations between elements and diseases. Few studies have examined trace elements in young people's hair. The objective of this study is to investigate the influence of age, gender and season on the contents of magnesium (Mg), calcium (Ca), Iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), selenium (Se) and strontium (Sr) as well as to establish the reference intervals (RIs).

Methods

We conducted a retrospective study of 589 apparently healthy children and adolescents under 19 years old. Quantitative analysis has been carried out using inductively coupled plasma-mass spectrometry (ICP-MS). Eight nutrient elements in peripheral blood of children and adolescents in eastern China were grouped according to age, sex or season, and analyzed using and Mann-Whitney U test and spearman statistical analyses. RIs were defined by using 95% confidence interval.

Results

Precisions of ICP-MS detecting for Mg, Ca, Fe, Cu, Zn, Mn, Se and Sr are 2.8%~12.2%. The linearity were all > 0.999 and the bias were all within 10%. Differences between contents of particularly Mg, Fe, Cu and Zn in girls' and boys' whole blood were found, and higher contents of Mg, Cu for boys were measured in some age groups. Positive correlations for Fe, Zn, Se and Sr, while negative for Ca and Cu were found with age. And substantial differences between age groups were stated. In general, an increasing trend was found for bioelements (Fe, Zn,Se) both for girls and boys in all age groups, while for Ca and Cu changes were even decreasing for children and teenagers. The most frequently correlating element pairs were Fe-Zn, Mg-Se, and Fe-Se in five successive age groups. Lower contents of essential elements (Mg, Ca, Fe, Zn, Se) were found in summer comparing with other seasons. Finally, the reference interval of each element was initially established according to age and gender grouping.

Conclusions

The contents of elements in whole blood varies depending mainly on the gender and age of children and adolescents. Besides, season is also a factor that affects the contents of elements in the body. The reference intervals of elements in whole blood grouped by age and gender provide a reference basis for clinical diagnosis and treatment of element-related diseases.

Backgroud

The content and metabolism of constant and trace elements are closely related to the normal development and health of the human body. Nutrient elements such as magnesium (Mg), calcium (Ca), Iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), selenium (Se) and strontium (Sr) can affect the activity of a variety of enzymes, promote nucleic acid, amino acid metabolism and involved in protein synthesis [1-5]. The lack of these elements will cause physiological and pathological phenomena such as slowing down of body growth, reduced immune function, and decreased memory ability [6]. Elements excess are as equally harmful as deficiency. An excess of one element can lead to a relative lack of other elements. Excessive or deficient contents of elements in the body can cause diseases of different organ systems, and the severity of the disease is directly related to the degree of excess or deficiency [7-11].

Biological monitoring of trace element concentrations in various media such as blood urine or hair is of great importance. The content of trace elements in whole blood has become a very popular index to evaluate the nutritional status of body, which is better than hair, nails, serum and so on [12,13]. At present, most clinical laboratories use atomic absorption method to detect elements content, but this method has the disadvantages of testing fewer types of elements one time, longer detection time, and narrow linear range. Inductively coupled plasma mass spectrometry (ICP-MS) has a wide linear range, lower detection limit, high sensitivity, fast detection speed, and can detect trace elements at microgram or even lower levels in samples. It can detect multiple elements at the same time, so ICP-MS is gold standard for element detection [14,15].

The importance of constant and trace elements including their contributions for children's and adolescents' health and diseases has been increasingly recognized, and it has also caused a high degree of concern in the medical and nutritional communities [7,9,12,13]. In our clinical work, we found that the contents of elements in the venous blood of children and adolescents varied greatly in different age groups, and there are even differences in the levels of elements between boys and girls.

How to correctly assess the nutritional status of the body according to the content of the nutrient elements has important clinical significance. In this paper, we attempt to evaluate the concentrations of essential elements such as Mg, Ca, Fe, Cu, Zn, Mn, Se and Sr using ICP-MS in children's and adolescents' whole blood (girls and boys) in age groups corresponding to successive phases of human ontogenesis as well as to find the reference interval for children and adolescent in Shandong Province, east China.

Methods

Study design and patients selection

In total, we retrospectively collected 731 nutrient elements detection reports of children and adolescents from the Laboratory Information System of Shandong Provincial Hospital Affiliated to Shandong First Medical University (the provincial medical union base) from May 2020 to April 2021. Finally, 589 apparently healthy children and adolescents (366 boys, 223 girls) were screened for analyzing the nutrient elements in children and adolescent of Shandong Province, east China. Inclusion criteria: no complaints of discomfort, no abnormalities in physical examination, no acute or chronic diseases, and good physical development evaluation. Young people were divided into five successive age groups: Group1 <1 year (33 boys, 30 girls), Group2 <3 years (71 boys, 55 girls), Group3 <6 years (98 boys, 64 girls), Group4 <12 years (129 boys, 57 girls), Group5 <19 years (35 boys, 17 girls). According to the season, it is divided into 4 groups, spring (March to May, 96 boys, 56 girls, average age 5.55±3.79), summer (June to August, 100 boys, 69 girls, average age 4.69±4.37), autumn (September to November, 102 boys, 59 girls, average age 5.20±3.97), and winter (December to February, 68 boys, 39 girls, average age 5.31±4.12). This study was approved by the Medical Ethics Committee of Shandong First Medical University in accordance with the Declaration of Helsinki (ethical approval number is SWYX: No.2020-152) .

Laboratory evaluation

Peripheral blood samples were collected from children and adolescent on an empty stomach and stored at 4-8°C. Nutrient elements in whole blood were determined within one week using Agilent 7900 Inductively Coupled Plasma Mass spectrometry (ICP-MS) (Agilent, Tokyo, Japan). The ultrapure water (≥18.0MΩ.cm, 25°C) used in the experiment was prepared by Milli-Q ultrapure water meter (Millipore Corporation, USA). Whole blood element testing kit was used for measurement (LOT: WL191102; Baichen, Hangzhou, China). The performance evaluation is as follows: The matching calibrator (LOT: 20011401) were tested for 3 times for assessing the linearity. In accordance with the Clinical and Laboratory Standards Institute (CLSI) 15A-3, within-run Coefficient of variations (CVs) and total CVs were calculated in four replicates of two concentrations quality control products (LOT:2001140) every day for five consecutive days. Trace Elements Whole Blood L-2 RUO (LOT: 1702825; Seronor™, Norway), which have been analyzed in independent laboratories (ALS Scandinavia AB, Luleå, Sweden), was determined for estimation of bias. The internal standard recovery was within 80-120%.

Statistical analysis and assessment criterion

Statistical analyses were performed using GraphPad Prism Software Version 5.0 (La Jolla, CA, USA). Shapiro-Wilk normality test was used for normality test. Because not all of the data were normally distributed, we present all data as median (Q1, Q3) and use Mann-Whitney U test for comparison between groups. Spearman coefficient values for correlation of two statistical variables. All calculations for definition of reference intervals (RIs) were based on (CLSI) 2008 guidelines, document C28-A3 [16]. 95% confidence interval (for normally distributed data; P2.5 ~ P97.5 for non-normally distributed data) was used as RIs . All p-values were two-tailed and p-values less than 0.05 were considered significant.

Results

Performance evaluation of ICP-MS in detecting nutrient elements

Limit of detection (LOD), and limit of quantification (LOQ). The LOD and LOQ were determined through detection of reagent blank for at least 10 times for each analyte and calculate the standard deviation (SD). The LOD was defined as three times of SD while the LOQ was defined as ten times of SD. The LODs of Mg, Ca and Fe were 0.003, 0, and 0.006 mmol/L, Cu and Zn were 0 and 0 μmol/L, Mn, Se and Sr were 1.366, 0.695, and 0 μg/L; LOQs, 0.009, 0, and 0.021 mmol/L, 0 and 0 μmol/L, 4.552, 2.316, and 0 μg/L, respectively. (Supplemental Table 1)

Linearity. Linearity was evaluated via linear regression of the calibrator concentrations on the theoretical concentrations. The correlation coefficients (r^2) of linearity for Mg, Ca, Fe, Cu, Zn, Mn, Se and Sr were >0.999 (Supplemental Figure 1).

Precision. Precisions of Mg, Ca, Fe, Cu, Zn, Mn, Se and Sr were evaluated using two concentrations of quality controls (QC). The within-run CVs (2.8~7.7% for lower QC, 3.1~4.7% for higher QC) and totle CVs (3.6~12.2% for lower QC, 5.5~10.1% for higher QC) for eight nutrient elements were shown in Supplemental table 2 respectively.

Estimation of bia. Comparing with the analytical data of Seronorm™ Trace Elements Whole Blood L-2 RUO, bias of Mg, Ca, Fe, Cu, Zn, Mn, Se and Sr were all within 10% (Supplemental Table 3).

The content of nutrient elements in both girls' and boys' whole blood

The content of Ca, Mg, Zn, Cu, Fe, Pb, and Cd expressed as 25th, 50th (median) and 75th percentiles in children's and adolescents' whole blood were shown in Table 1, while the average and SD values were presented in Figure 1. Higher contents of Mg, Fe, Cu, Zn were detected in boys' whole blood, in comparison to girls'. And there were also differences of Mg, Cu and Mn between boys and girls in <6 years groups, <12 years groups and <19 years groups, respectively.

In the individual age groups, the differences related to content of elements in

whole blood of tested children and adolescents

As shown in Table 2, on the basis of the Spearman test, we found positive correlations for Fe, Zn, Se and Sr, while negative for Ca and Cu in girls' or (and) boys' whole blood. However, in case of Mg and Mn, there are no statistically significant correlations between their content and age of the children and adolescents. While the change trend of each nutrient element in differernt age groups were presented intuitively in Figure 1. The content of Fe, Zn and Se in girls or (and) boys increased with age, while the content of Ca decreased with age. The content of Cu increased first and decreasd subsequently with age. But there were no obvious change trend of Mg ,Mn and Sr. Mann-Whitney U test was applied to establish whether there were statistically significant differences of the nutrient elements between individual age groups. Results of the analysis point to major significant differences in Ca, Fe and Zn contents between individual age groups, both for girls' and boys' whole blood (Table 3).

Relationships between element contents in children's and adolescents' peripheral blood.

Spearman test revealed a largest number of correlations between nutrient elements in boys and girls of different age groups. Positive correlations between Fe and Zn (except for the <6 years and <19 years group in girls), Mg and Se (except for the <3 years and <12 years group in girls), and Fe and Se (except for the <6 years and <19 years group in girls) are observed. The infrequent negative correlations have been found only for Cu and Sr (<3 years group in boys), for Se and Sr (<19 years group in girls).

In different seasons, the differences of element contents in both girls' and boys' blood

There are seasonal variation of each element visually shown in Figure 2. Various elements (Mg, Ca, Fe, Zn, Se) performed a low concentration trend in girls' or (and) boys' whole blood in summer comparing with in winter.

Reference intervals for eight nutrient elements in children and adolescent.

Based on the above investigated data, preliminary RIs for each element were established in table 5 based on age and gender. There are some observable differences among different age groups or between boys and girls.

Discussion

The importance of elements for human diseases has been increasingly recognized, including their contribution to the growth and development of children and adolescents [17,18]. In this study, we investigated the contents of eight nutrient elements in peripheral blood of children and adolescents using ICP-MS, and analyzed the differences and correlations between elements and gender, age or season. Then reference interval of each element was established according to age and gender.

Dynamic changes regarding weight and growth gain happens in children and adolescents. Trace and constant elements plays an important role in growth and development. According to the special functions of different nutrient elements in the human body, the detection of functional indicators such as certain enzyme activities can also indirectly reflect the nutritional status of human elements, such as ceruloplasmin and selenoprotein P [19,20]. However, these biological indicators change only when the element is severely deficient. Therefore, early and accurate detection of elements deficiency is very important. The content of elements in biological samples is extremely tiny, and accurate measurement requires high-precision instruments. The performance evaluation results of ICP-MS for detecting nutrient elements showed high sensitivity, high precision, high accuracy, and wide linear range. So the use of Baichen kit to detect nutrient elements on ICP-MS can fully meet the clinical requirements. In addition, the accurate detection of nutrient elements also needs to exclude contamination and other influencing factors during the collection, storage, and testing of samples.

According to our statistical results, the content of nutrient elements in whole blood has a certain relationship with age, gender and season. On the one hand, it may be that different growth stages have different requirements for different nutrient elements. On the other hand, it may indicate that a certain element is lacking in a certain age group. The content of Ca had a downward trend with age and was negatively correlated with age. High levels of Ca in infants and toddlers must be related to supplementation of vitamin D after birth to 2-3 years old. Thus, preschool, school-age children and teenagers especially need to pay attention to calcium supplementation and take more time for outdoor activity. The content of Fe increases with age except for girls from <12 age group to <19 age group, it should be the cause of menstrual cramps in teenage. For the highly positive correlation with Fe and Zn, Zn had the same change trend with Fe. So adolescent girls should take extra iron and zinc supplements. Sr has a large range of variation, which is most likely related to the habit of drinking pure water or tap water. Most of the elements present a high level in autumn and winter, generally due to the geographical and custom characteristics, as hot climate, more sweat and vigorous metabolism of the body, the reduction of outdoor activities in summer and the habit of tonic in autumn and winter, so it is especially necessary to supplement nutrients in summer.

There are also undesirable and unjustified aspects of this research. When analyzing the correlations between the elements, it was found that there were more related elements in boys than that in girls, which may be due to the small number of girls in the study. Multi-center studies are the best choice to obtain valuable RIs for nutrient elements. We analyzed the levels of elements in whole blood of children and adolescents in Shandong Province representing east China by ICP-MS considering gender, age and season. A wider area and a larger population of young people will have more clinical value. And various environmental influences can also be considered as influencing factors.

Deficiencies of nutrient elements have many long-term adverse effects on children's growth and development. In addition to early detection and intervention, it is more ideal to take effective measures to prevent children and adolescents from nutritional deficiencies [21]. Preventive supplementation of nutrient elements emphasizes low dose and long-term supplementation, while treatment of deficiency emphasizes adequate amount and correction in a short time as soon as possible, so as to reduce adverse effects on the growth and development of children and adolescents. Long-term follow-up and regular monitoring are needed to prevent the recurrence of nutrient deficiency [22].

Conclusions

In conclusion, by analyzing the contents of nutrient elements in whole blood of apparently healthy children and adolescents using ICP-MS, differences in elements' contents were found to be dependent on gender and age, the strongest correlations are observed for Fe-Zn, Mg-Se and Fe-Se pairs both for girls and boys. Reference intervals worked out for elemental contents is hoped to be helpful for clinical assessment for children's and adolescents' development and health care.

Abbreviations

ICP-MS: inductively coupled plasma-mass spectrometry; RIs: Reference intervals; Mg: magnesium; Ca: calcium; Fe: Iron; Cu: copper; Zn: zinc; Mn: manganese; Se: selenium; Sr: strontium; CLSI: Clinical and Laboratory Standards Institute; CVs: Coefficient of variations; LOD: Limit of detection; LOQ: limit of quantification; SD: standard deviation; QC: quality controls

Declarations

Acknowledgments: Authors thank all those who participated in this study.

Ethics approval and consent to participate: This study was approved by the Medical Ethics Committee of Shandong First Medical University in accordance with the Declaration of Helsinki (ethical approval number is SWYX: No.2020-152) .

Consent for publication: Participants were provided a study overview and verbal consent was attained.

Availability of data and materials: The datasets used and analyzed in this study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: FL and YW conceived and designed study. SS, YC, YX, XJ, XG and SC contributed to acquisition of data. FL, BL and YW analyzed the data and wrote the paper. All authors assisted in revising the text and approved the final manuscript.

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Tables

TABLE 1 Percentile and median values of content of elements in girls' and boys' whole blood according to age group

Boys	Mg	Ca	Fe	Cu	Zn	Mn	Se	Sr	Girls	Mg	Ca	Fe	Cu	Zn
(N=366)									**(N=223)**					
Minimum	1.18	1.18	3.72	9.80	18.72	0.84	73.17	8.07		1.05	1.25	3.95	7.02	17.35
25% Percentile	1.62	1.58	6.28	14.56	64.89	8.83	140.80	22.32		1.60	1.59	6.08	13.79	62.30
Median	1.72*	1.66	6.73**	16.47**	77.72**	11.20	162.30	29.27**		1.69*	1.68	6.50**	15.74**	72.50**
75% Percentile	1.84	1.76	7.25	18.41	87.80	13.57	185.90	36.27		1.78	1.78	7.10	17.07	84.78
Maximum	2.52	2.27	10.03	28.49	138.70	31.88	472.90	81.23		2.13	2.08	8.92	23.90	119.10
<1 (N=33)									**<1 (N=30)**					
Minimum	1.18	1.59	4.25	10.30	18.72	0.84	73.17	8.07		1.37	1.65	4.50	7.57	17.35
25% Percentile	1.57	1.72	5.33	14.74	38.29	8.39	106.70	14.65		1.53	1.76	5.30	12.68	32.91
Median	1.64	1.82	5.76	16.62	50.86	12.45	130.00	23.48		1.64	1.86	5.79	15.73	47.77
75% Percentile	1.80	1.93	6.20	19.64	59.92	15.52	142.00	30.21		1.77	1.96	6.26	17.00	65.03
Maximum	1.97	2.07	6.94	27.44	84.36	24.93	212.60	81.23		2.05	2.05	7.67	23.27	82.08
<3 (N=71)									**<3 (N=55)**					
Minimum	1.32	1.30	5.22	10.94	39.94	3.44	76.83	14.11		1.50	1.49	4.40	12.76	43.35
25% Percentile	1.63	1.64	6.12	15.69	55.14	9.81	141.60	20.83		1.64	1.66	5.97	16.19	53.29
Median	1.71	1.73	6.48	17.73	60.85	12.14	159.40	24.43		1.71	1.74	6.35	16.81	64.62
75% Percentile	1.83	1.81	6.89	20.01	69.75	13.44	176.60	32.04		1.81	1.82	6.93	18.58	76.39
Maximum	2.26	2.06	8.57	28.49	123.50	25.34	212.70	53.76		2.08	2.08	8.47	23.90	103.50
<6 (N=98)									**<6 (N=64)**					
Minimum	1.37	1.34	4.66	11.56	47.19	3.84	110.90	12.34		1.05	1.25	3.95	10.74	42.80
25% Percentile	1.64	1.58	6.32	15.47	71.22	8.52	146.10	22.66		1.58	1.58	6.14	14.27	67.31
Median	1.72**	1.65	6.78	16.90***	78.68	10.46*	162.90	28.99		1.67**	1.67	6.53	15.52***	74.51
75% Percentile	1.84	1.72	7.22	18.73	85.64	12.81	186.60	36.76		1.77	1.75	7.08	17.29	83.44
Maximum	2.28	1.98	8.10	22.88	112.40	26.23	233.60	58.55		2.13	1.90	8.40	23.22	111.10
<12 (N=129)									**<12 (N=57)**					
Minimum	1.36	1.18	4.11	9.82	46.68	2.14	98.26	14.54		1.32	1.35	5.62	7.02	50.78
25% Percentile	1.62	1.57	6.49	14.40	75.95	8.90	147.80	24.52		1.64	1.54	6.49	13.28	70.81
Median	1.72	1.64	6.88	16.25**	82.19	11.38	167.30	30.72		1.74	1.62	7.02	14.69**	81.74
75% Percentile	1.83	1.70	7.36	17.54	90.67	13.38	190.90	36.42		1.79	1.67	7.24	16.45	90.09
Maximum	2.52	2.04	9.36	28.17	117.10	31.88	472.90	78.43		2.07	1.82	8.92	23.06	119.10
<19 (N=35)									**<19 (N=17)**					
Minimum	1.35	1.23	3.72	9.80	65.00	3.64	89.84	14.62		1.47	1.37	5.12	10.31	56.89
25% Percentile	1.64	1.47	6.79	12.36	81.82	8.93	142.40	24.59		1.59	1.49	6.52	11.28	74.08
Median	1.79*	1.54	7.55	13.56	94.28	11.01	163.00	34.97		1.67*	1.57	7.11	12.79	86.47
75% Percentile	1.93	1.61	8.19	14.67	105.70	14.56	186.40	42.12		1.74	1.61	7.60	14.62	94.66

Maximum	2.13	2.27	10.03	23.09	138.70	31.19	223.80	70.41		1.95	1.90	7.86	16.73	117.30

Significant differences of elements' amount in whole blood between boys and girls (*p < 0.05, **p < 0.01, ***p < 0.001)

TABLE 2 Spearman coefficient values for correlation between content of elements in whole blood and age of all girls and boys

	Mg	Ca	Mn	Fe	Cu	Zn	Se	Sr
All	0.08	-0.53****	-0.05	0.45****	-0.31****	0.62****	0.26****	0.26****
Boys	0.08	-0.46****	-0.02	0.43****	-0.33****	0.63****	0.23****	0.28****
Girls	0.04	-0.62****	-0.06	0.46****	-0.34****	0.59****	0.34****	0.24***

Significant correlations of elements in whole blood and age (***p< 0.001 ****p< 0.0001)

TABLE 3 Results of the significant differences of nutrient elements contents between individual age groups in whole blood for boys and girls respectively.

	<1 (a)		**<3 (b)**		**<6 (c)**		**<12 (d)**		**<19 (e)**	
	Boys	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**
Mg	c*,d*,e*	b*,d*	c*	a*	a*	d*	a*	a*,c*	a*	—
Ca	b**,c***, d***,e***	b***,c***, d***,e***	a**,c***, d***,e***	a***,c**, d***,e***	a***,b***, e***	a***,b**, d**,e**	a***,b***, e***	a***,b***,c**	a***,b***, c***,d***	a***,b***,c
Fe	b***,c***, d***,e***	b***,c***, d***,e***	a***,c*, d***,e***	a***,d***, e**	a***,b*, e***	a***,d*, e*	a***,b***, e***	a***,b***,c*	a***,b***, c***,d***	a***,b**, c
Cu	e***	b**,e*	d***,e***	a**,c***, d***,e***	d**,e***	b***,d*, e***	b***,c**, e***	b***,c*, e**	a***,b***, c***,d***	a*,b***, c***,d**
Zn	b***,c***, d***,e***	b***,c***, d***,e***	a***,c***, d***,e***	a***,c***, d***,e***	a***,b***, d***,e***	a***,b***,d*,e**	a***,b***, c***,e***	a***,b***,c*	a***,b***, c***,d***	a***,b***,c
Mn	—	—	c*	—	b*	—	—	—	—	—
Se	b***,c***, d***,e***	b**,c***, d***,e**	a***,d**	a**.d**	a***,	a***	a***,b**	a***,b**	a***,	a**
Sr	c**,d***, e***	d*	c*,d***, e***	c**,d***, e**	a**,b*, e*	b**	a***,b***	b***	a***,b***	b**

Significant differences of elements' amount in boys' and girls' whole blood between two age groups (*p < 0.05, **p < 0.01, ***p < 0.001,****p < 0.0001)

TABLE 4 Statistically significant correlations between contents of elements in girls' and boys' whole blood in successive age groups and corresponding values of Spearman correlation coefficients

	<1		<3		<6		<12		<19	
Boys										
	Mg-Fe***	0.58	Mg-Ca**	0.34	Mg-Ca***	0.34	Mg-Ca*	0.22	Mg-Fe***	0.75
	Mg-Cu*	0.41	Mg-Fe***	0.54	Mg-Fe***	0.48	Mg-Fe***	0.43	Mg-Zn**	0.49
	Mg-Zn*	0.43	Mg-Zn*	0.25	Mg-Cu**	0.31	Mg-Cu***	0.36	Mg-Se**	0.49
	Mg-Se**	0.45	Mg-Se**	0.35	Mg-Zn***	0.44	Mg-Zn**	0.28	Ca-Cu**	0.49
	Ca-Mn**	0.45	Ca-Mn*	0.27	Mg-Se**	0.30	Mg-Se*	0.21	Fe-Zn**	0.49
	Fe-Cu**	0.45	Ca-Cu***	0.40	Ca-Cu**	0.30	Ca-Mn*	0.22	Fe-Se*	0.37
	Fe-Zn*	0.44	Fe-Zn***	0.39	Ca-Se*	0.22	Ca-Cu***	0.29	Zn-Se***	0.58
	Fe-Se***	0.61	Fe-Se***	0.56	Mn-Fe**	0.31	Mn-Zn*	0.18		
	Cu-Zn**	0.48	Cu-Sr*	-0.26	Fe-Zn***	0.47	Fe-Zn***	0.39		
	Cu-Se*	0.42	Zn-Se**	0.35	Fe-Se*	0.23	Fe-Se***	0.42		
	Zn-Se*	0.40								
Girls										
	Mg-Cu***	0.58	Fe-Zn***	0.49	Mg-Ca*	0.26	Mg-Ca**	0.37	Mg-Cu*	0.49
	Mg-Se*	0.44	Fe-Se***	0.51	Mg-Mn*	0.27	Mg-Fe**	0.41	Mg-Se**	0.71
	Ca-Mn*	0.41	Zn-Se*	0.33	Mg-Fe***	0.46	Mg-Cu**	0.42	Se-Sr*	-0.55
	Fe-Cu**	0.54			Mg-Zn***	0.47	Mg-Zn*	0.30		
	Fe-Zn*	0.42			Mg-Se***	0.51	Ca-Cu***	0.44		
	Fe-Se**	0.53					Ca-Sr*	0.31		
	Cu-Zn***	0.71					Fe-Zn***	0.43		
							Fe-Se*	0.30		
							Zn-Se*	0.31		

Significant correlations of two elements (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$)

TABLE 5 Reference intervals for nutrient elements in whole blood of successive age groups and gender groups

	<1 (N=63)	<3 (N=126)	<6 (N=162)	<12 (N=186)	<19 (N=52)	Boy (N=366)	Girl (N=223)
Mg	1.33-1.97	1.43-2.08	1.45-2.11	1.45-2.11	1.38-2.11	1.43-2.12	1.44-2.05
Ca	1.60-2.08	1.48-2.00	1.42-1.90	1.40-1.83	1.23-2.27	1.40-2.02	1.40-1.97
Fe	4.43-7.10	5.22-8.30	5.29-8.08	5.64-8.33	5.23-9.39	5.22-8.47	5.02-8.09
Cu	8.66-23.68	12.29-23.90	12.72-22.51	10.13-22.62	9.80-23.09	10.94-23.58	10.03-22.83
Zn	17.37-79.77	43.35-101.49	52.32-102.34	58.24-106.74	61.00-124.50	41.07-111.59	36.19-107.69
Mn	1.76-17.32	3.68-22.66	4.50-19.16	4.03-20.24	3.64-31.19	3.84-22.19	4.02-20.16
Se	69.11-195.49	104.69-211.91	113.29-224.73	111.73-239.74	103.46-234.94	98.26-218.83	93.19-236.41
Sr	9.1-81.23	14.11-56.18	13.81-54.13	16.21-56.56	10.82-57.74	13.74-55.55	14.51-59.29

Figures

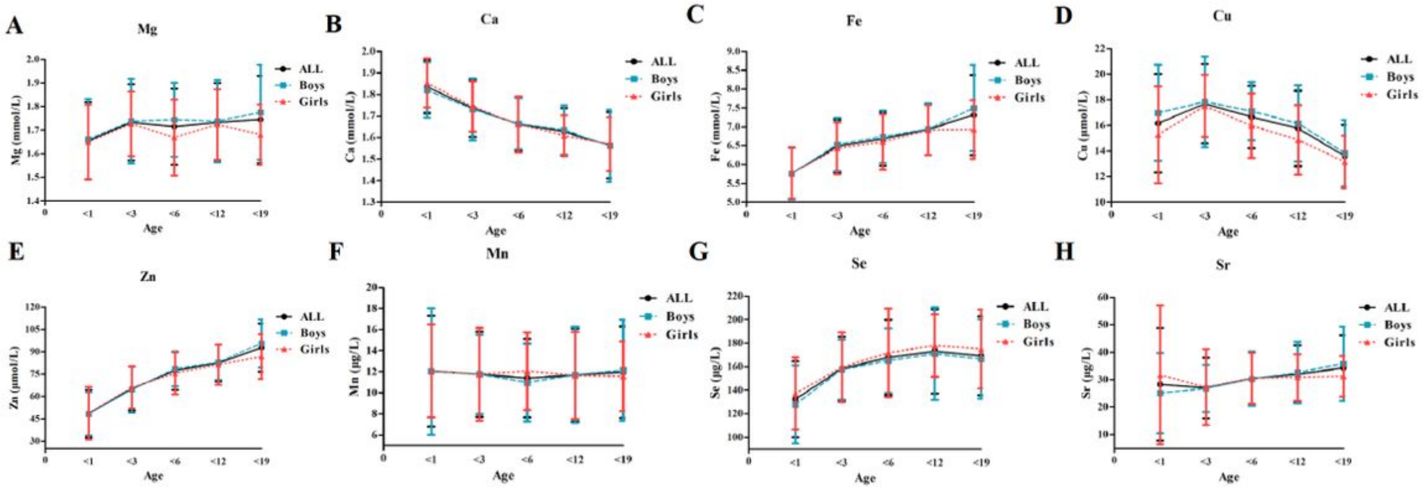

Figure 1

The change trend of each nutrient element in successive age groups. Line charts were used to visually display the changes in the contents of various elements in the peripheral whole blood of boys or (and) girls with age. Data were expressed as means ± standard deviation. Significant differences of elements contents between age groups were listed in Table 3.

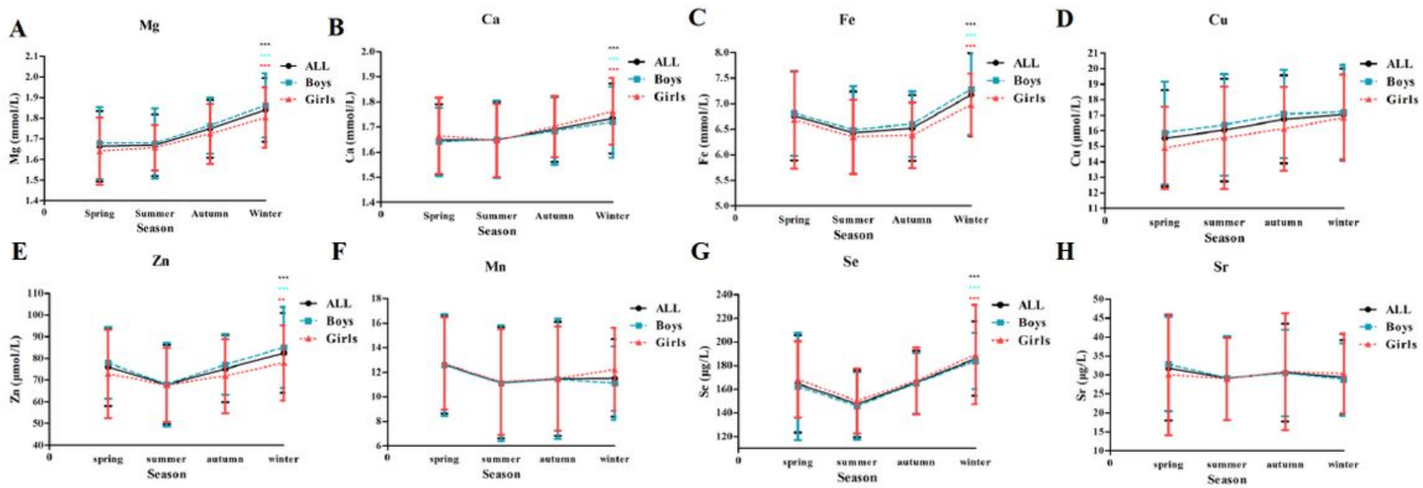

Figure 2

The change trend of each nutrient element in successive seasons. Line charts were used to visually display the changes in the contents of various elements in the peripheral whole blood of boys or (and) girls with season. Data were expressed as means ± standard deviation. Elements (Mg, Ca, Fe, Zn, Se) contents were higher in winter than that in summer (Zn for Girls: $^{**}p < 0.01$; other elements for All, Boys, Girls: $^{***}p < 0.001$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementFigure1.jpg
- supplementtable1.doc
- supplementtable2.doc
- supplementtable3.doc