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Analysis of Eight Nutrient Elements in Whole Blood of Children and Adolescents Using Inductively Coupled Plasma-Mass Spectrometry

Fang Luan[1] · Bin Liu[2] · Shuzhen Sun[3] · Yuan Chen[3] · Yanqiu Xu[1] · Xuerui Jiang[1] · Xiangrui Guo[1] · Shiqing Cheng[1] · Yong Wang[1]

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Abstract

Few researches have been conducted on elements in whole blood of young people. Our study was 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 reference intervals (RIs). We conducted a retrospective study of 589 apparently healthy children and adolescents. Quantitative analysis had been carried out using inductively coupled plasma-mass spectrometry (ICP-MS). Test results were analyzed using and MannWhitney U test, Spearman and Pearson statistical analyses. RIs were defined by using 95% confidence interval. Differences between contents of Mg, Fe, Cu, and Zn in girls' and boys' whole blood were found. Positive correlations for Fe, Zn, Se, and Sr, while negative for Ca and Cu were found with age. Increasing trends were found for Fe, Zn, and Se, while for Ca and Cu, changes were even decreasing for children and teenagers. The most frequently correlating element pairs were FeZn, MgSe, and FeSe in five successive age groups. Lower contents of Mg, Ca, Fe, Zn, and Se were found in summer. Finally, the reference interval of each element was initially established according to age and gender grouping. The contents of elements in whole blood vary depending mainly on the gender and age of children and adolescents. 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.

Keywords Children and adolescents · Nutrient elements · Whole blood · ICP-MS · Reference intervals

Introduction

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 are 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].

Many factors affect the content of elements in the blood, urine, hair or nails and biological monitoring of trace element concentrations in various media is of great importance. Thus, it is difficult to unequivocally assess which of the indicators is the best. Actually, complementary element analysis in many media (including 24-h urine collection) allows the assessment of element level in the body. The content of elements in the hair or nails may not be good indicators for

✉ Yong Wang
404389139@qq.com

[1] Department of Clinical Laboratory, Shandong Provincial Hospital Affiliated to Shandong First Medical University, No. 324 Jingwu Road, Jinan, Shandong 250021, China

[2] Department of Biomedical Engineering, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong, China

[3] Department of Pediatry, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong, China

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many factors' affection. Retention of 24-h urine is time consuming and laborious, which is difficult to collect for infants. In comparison, 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 the hair, nails, serum, and so on [12, 13].

At present, most clinical laboratories use atomic absorption method to detect element 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 (infant stage, toddler stage, preschool stage, school age stage, adolescent stage) of human ontogenesis as well as to find the reference interval for children and adolescents in Shandong Province, east China.

Methods

Study Design and Patients' Selection

In total, we retrospectively collected 731 nutrient element 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 include the following: 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 as follows: 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), and 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. 2020152).

Laboratory Evaluation

Peripheral blood samples were collected from children and adolescents 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℃) used in the experiment was prepared by a Milli-Q ultrapure water meter (Millipore Corporation, USA). Whole blood element testing kit was used for measurement (LOT: WL191102; Baiqin, 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 of 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 80120%.

Statistical Analysis and Assessment Criterion

Statistical analyses were performed using GraphPad Prism Software Version 5.0 (La Jolla, CA, USA). ShapiroWilk 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 MannWhitney U test for comparison between groups. Spearman or Pearson coefficient values for correlation of two statistical variables were also determined. All calculations for definition of reference intervals (RIs) were based on CLSI document EP28-A3c (2008). The 95% confidence interval ($\bar{x} \pm 1.96s$ for normally distributed data; $P_{2.5} \sim P_{97.5}$ for non-normally distributed data) was used as RIs [16]. 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 the standard deviation (SD) was calculated. 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 Fig. 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 total CVs (3.6 ~ 12.2% for lower QC, 5.5 ~ 10.1% for higher QC) for eight nutrient elements are shown in Supplemental Table 2, respectively.

Estimation of Bias

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 was all within 10% (Supplemental Table 3).

The Content of Nutrient Elements in Both Girls' and Boys' Whole Blood

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

In the Individual Age Groups, the Differences Related to Contents 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. Meanwhile, the change trend of each nutrient element in different age groups is presented intuitively in Fig. 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 decreased subsequently with age. But there were no obvious change trend of Mg, Mn, and Sr. MannWhitney U test was applied to establish whether there were statistically significant differences of the nutrient elements between the individual age groups. Results of the analysis pointed to major significant differences in Ca, Fe, and Zn contents between the individual age groups, both for girls' and boys' whole blood (Table 3).

Relationships Between Element Contents in Children's and Adolescents' Peripheral Blood

Spearman or Pearson test revealed a large number of correlations between nutrient elements in boys and girls of different age groups. Positive correlations between Fe and Zn (except for the < 6-year and < 19-year groups in girls), Mg and Se (except for the < 3-year and < 12-year groups in girls), and Fe and Se (except for the < 6-year and < 19-year groups in girls) are frequently observed. The infrequent negative correlations have been found only for Cu and Sr (< 3-year group in boys), for Se and Sr (< 19-year group in girls) (Table 4).

In Different Seasons, the Differences of Element Contents in Both Girls' and Boys' Blood

There are seasonal variations of each element visually shown in Fig. 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 are established in Table 5 based on age and gender. There are some observable differences among different age groups or between boys and girls.

Table 1 Percentile and median values of content of elements (μg/L) 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	Mn	Se	Sr
(N = 366)									(N = 223)								
Minimum	28,320	47,200	208,320	627.20	1216.80	0.84	73.17	8.07		25,200	50,000	221,200	449.28	1127.75	1.46	80.37	10.39
25% percentile	38,880	63,200	351,680	931.84	4217.85	8.83	140.80	22.32		38,400	63,600	340,480	882.56	4049.5	9.33	147.40	22.10
Median	41,280*	66,400	376,880**	1054.08**	5051.80**	11.20	162.30	29.27**		40,560*	67,200	364,000**	1007.36**	4712.5**	11.33	165.80	27.84**
75% percentile	44,160	70,400	406,000	1178.24	5707.00	13.57	185.90	36.27		42,720	71,200	397,600	1092.48	5510.7	14.24	183.90	34.79
Maximum	60,480	90,800	561,680	1823.36	9015.50	31.88	472.90	81.23		51,120	83,200	499,520	1529.6	7741.5	29.61	367.80	122.60
< 1 (N = 33)									< 1 (N = 30)								
Minimum	28,320	63,600	238,000	659.20	1216.80	0.84	73.17	8.07		32,880	66,000	252,000	484.48	1127.75	1.81	80.37	12.66
25% percentile	37,680	68,800	298,480	943.36	2488.85	8.39	106.70	14.65		36,720	70,400	296,800	811.52	2139.15	8.48	111.30	18.15
Median	39,360	72,800	322,560	1063.68	3305.90	12.45	130.00	23.48		39,360	74,400	324,240	1006.72	3105.05	12.63	141.50	22.90
75% percentile	43,200	77,200	347,200	1256.96	3894.80	15.52	142.00	30.21		42,480	78,400	350,560	1088	4226.95	15.07	163.60	34.46
Maximum	47,280	82,800	388,640	1756.16	5483.40	24.93	212.60	81.23		49,200	82,000	429,520	1489.28	5335.2	20.16	184.80	122.60
< 3 (N = 71)									< 3 (N = 55)								
Minimum	31,680	52,000	292,320	700.16	2596.10	3.44	76.83	14.11		36,000	59,600	246,400	816.64	2817.75	1.46	93.19	10.39
25% percentile	39,120	65,600	342,720	1004.16	3584.10	9.81	141.60	20.83		39,360	66,400	334,320	1036.16	3463.85	9.44	135.70	19.25
Median	41,040	69,200	362,880	1134.72	3955.25	12.14	159.40	24.43		41,040	69,600	355,600	1075.84	4200.3	11.33	161.80	22.82
75% percentile	43,920	72,400	385,840	1280.64	4533.75	13.44	176.60	32.04		43,440	72,800	388,080	1189.12	4965.35	13.50	181.10	31.32
Maximum	54,240	82,400	479,920	1823.36	8027.50	25.34	212.70	53.76		49,920	83,200	474,320	1529.6	6727.5	24.77	252.20	83.34
< 6 (N = 98)									< 6 (N = 64)								
Minimum	32,880	53,600	260,960	739.84	3067.35	3.84	110.90	12.34		25,200	50,000	221,200	687.36	2782	1.86	91.75	14.66
25% percentile	39,360	63,200	353,920	990.08	4629.30	8.52	146.10	22.66		37,920	63,200	343,840	913.28	4375.15	9.87	153.80	23.39
Median	41,280**	66,000	379,680	1081.60***	5114.20	10.46*	162.90	28.99		40,080**	66,800	365,680	993.28***	4843.15	11.41*	168.30	29.13
75% percentile	44,160	68,800	404,320	1198.72	5566.60	12.81	186.60	36.76		42,480	70,000	396,480	1106.56	5423.60	14.39	190.50	36.14
Maximum	54,720	79,200	453,600	1464.32	7306.00	26.23	233.60	58.55		51,120	76,000	470,400	1486.08	7221.50	24.91	367.80	54.74
< 12 (N = 129)									< 12 (N = 57)								
Minimum	32,640	47,200	230,160	628.48	3034.20	2.14	98.26	14.54		31,680	54,000	314,720	449.28	3300.70	2.92	126.00	15.00
25% percentile	38,880	62,800	363,440	921.60	4936.75	8.90	147.80	24.52		39,360	61,600	363,440	849.92	4602.65	8.68	161.50	25.43
Median	41,280	65,600	385,280	1040**	5342.35	11.38	167.30	30.72		41,760	64,800	393,120	940.16**	5313.10	11.38	176.80	29.43
75% percentile	43,920	68,000	412,160	1122.56	5893.55	13.38	190.90	36.42		42,960	66,800	405,440	1052.80	5855.85	14.44	191.90	36.07
Maximum	60,480	81,600	524,160	1802.88	7611.50	31.88	472.90	78.43		49,680	72,800	499,520	1475.84	7741.50	29.61	255.10	63.13
< 19 (N = 35)									< 19 (N = 17)								
Minimum	32,400	49,200	208,320	627.20	4225.00	3.64	89.84	14.62		35,280	54,800	286,720	659.84	3697.85	6.97	116.10	15.79
25% percentile	39,360	58,800	380,240	791.04	5318.30	8.93	142.40	24.59		38,160	59,600	365,120	721.92	4815.20	8.90	148.00	26.15
Median	42,960*	61,600	422,800	867.84	6128.20	11.01	163.00	34.97		40,080*	62,800	398,160	818.56	5620.55	10.37	171.60	30.46
75% percentile	46,320	64,400	458,640	938.88	6870.50	14.56	186.40	42.12		41,760	64,400	425,600	935.68	6152.90	13.80	203.20	37.15
Maximum	51,120	90,800	561,680	1477.76	9015.50	31.19	223.80	70.41		46,800	76,000	440,160	1070.72	7624.50	18.62	236.40	42.89

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

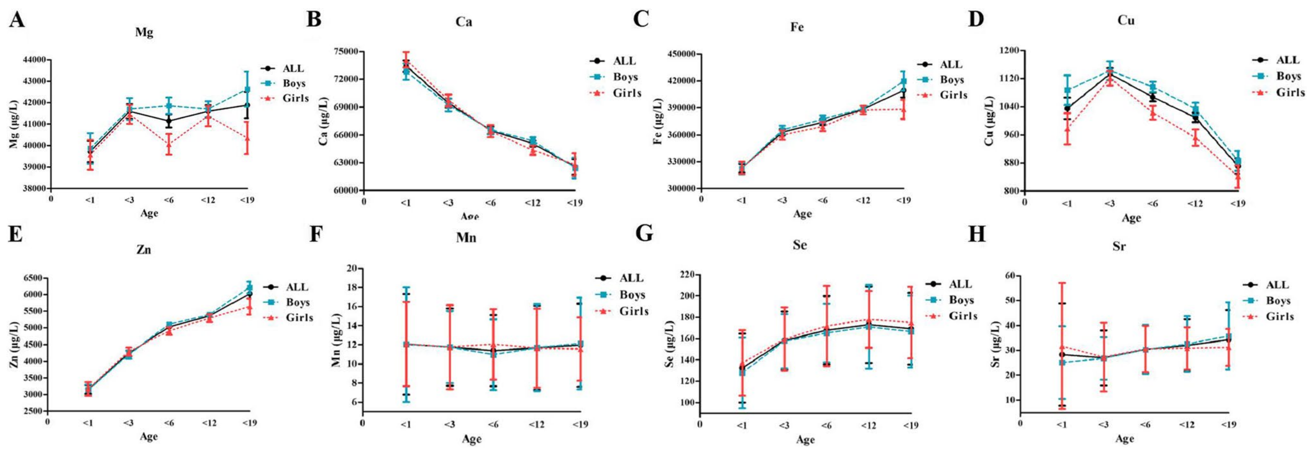

Fig. 1 The change trend of each nutrient element (μg/L) in successive age groups. Line charts were used to visually display the changes in the contents of various elements in the peripheral whole blood of boys or (and) girls with age. Data were expressed as means ± standard deviation. Significant differences of element contents between age groups are listed in Table 3

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 (***$p < 0.001$, **** $p < 0.0001$)

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 whole blood of children and adolescents using ICP-MS, and analyzed the differences and correlations between elements according to gender, age or season. Then, reference interval of each element was established according to age and gender.

Dynamic changes regarding weight and growth gain happen in children and adolescents. Trace and constant elements play 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 element 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, and high accuracy. So the use of Baiqin 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 habits of vitamin D after birth to 23 years old. Thus, preschool, school-age children, and teenagers especially need to pay attention to calcium supplementation and take more time for outdoor activities. The content of Fe increases with age except for girls from the < 12 age group to the < 19 age group; it should be the cause of menstrual cramps in teenagers. For the highly positive correlation of Fe and Zn, Zn has the same change trend with Fe. So adolescent girls should take extra iron and zinc supplements.

Table 3 Results of the significant differences of nutrient element 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 (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$)

Both Mn and Sr have large ranges of variation, which are most likely related to the habit of drinking and diet. Most of the elements present a higher level in autumn and winter, generally due to the geographical and custom characteristics, such 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.

Maria Długaszek et al. reported the inter-element interactions in human hair [13]. Thus, we analyzed the correlations between the elements in whole blood. More positive correlations were found between Fe and Zn, Mg, and Se. The interactions may be due to the similar physicochemical properties of the elements and the similar size of atoms [13, 21]. In case of Cu and Sr, the negative correlation may be metallic elements competing for binding sites in biomolecules [21]. The correlations between two elements can reflect mutual synergic or antagonistic interactions between them during the absorption process, transmembrane transport, accumulation, and excretion phases [13]. It was found that there were more related elements in boys than that in girls, which may be due to the smaller number of girls in the study.

Multi-center studies are the best choice to obtain valuable RIs. As to the particularity of nutritional elements affected by multiple factor (age, gender, season, region, and environment), we analyzed the levels of elements in whole blood of children and adolescents in Shandong Province representing east China by ICP-MS. Sample size is really an important issue for establishing the reference interval, and a larger population of young people will have more clinical value. According to CLSI C28-A3, the minimum sample size for newborns and infants is 20 cases, but it is recommended to recruit at least 40 cases [16, 22]. Thirty three boys and 30 girls were included in Group < 1 year. So the sample size was adequate. Thus, there may be other reasons for the broad reference ranges of Mn and Sr (in the < 1 group). Mn and Sr are indispensable trace elements and mainly ingested by the human body through food and water. Mn is abundant in cereals and low in meat. Ordinary drinking water or pure water contains very little Sr [23–25]. So, eating and drinking habits have a huge impact on these two elements in the body. The Mn and Sr levels of an infant (< 1 year) a strongly linked to how he or she is fed. Our investigations find that there are big differences in the feeding patterns of infants < 1 year old. The differences are as follows: artificial feeding (differences in brands of milk powder), breastfeeding (differences in mother's diet), birth season (differences in seasonal food), and the time to add supplementary food and so on.

The reference range of nutrient elements in whole blood of children and adolescents obtained in this study varied from the current reference range used in lab (supplied by the third-party clinical testing organization Hangzhou Baichen Medical Laboratory Co., Ltd.). The content of elements

Table 4 Statistically significant correlations between contents of elements in girls' and boys' whole blood in successive age groups and corresponding values of Spearman or Pearson 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–Ca*	0.51
	Mg–Se*	0.44	Fe–Se***	0.51	Mg–Mn*	0.27	Mg–Fe**	0.41	Mg–Se**	0.62
	Ca–Mn*	0.41	Zn–Se*	0.33	Mg–Fe***	0.46	Mg–Cu**	0.42	Zn–Se*	0.52
	Fe–Cu**	0.54			Mg–Zn***	0.47	Mg–Zn*	0.30	Se–Sr**	−0.63
	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 (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$)

does change and fluctuate with age and sex, so the reference range should be evaluated by population. In China, the living conditions and dietary habits vary greatly among regions. In addition to age and sex, the daily diet or nutrient intake information of the population is also important. According to the Report on Nutrition and Health of Children and Adolescents in China, Shandong Province took the lead in participating in the nutrition improvement plan for compulsory education students, so the nutrition of children and adolescents in this research area is relatively reasonable.

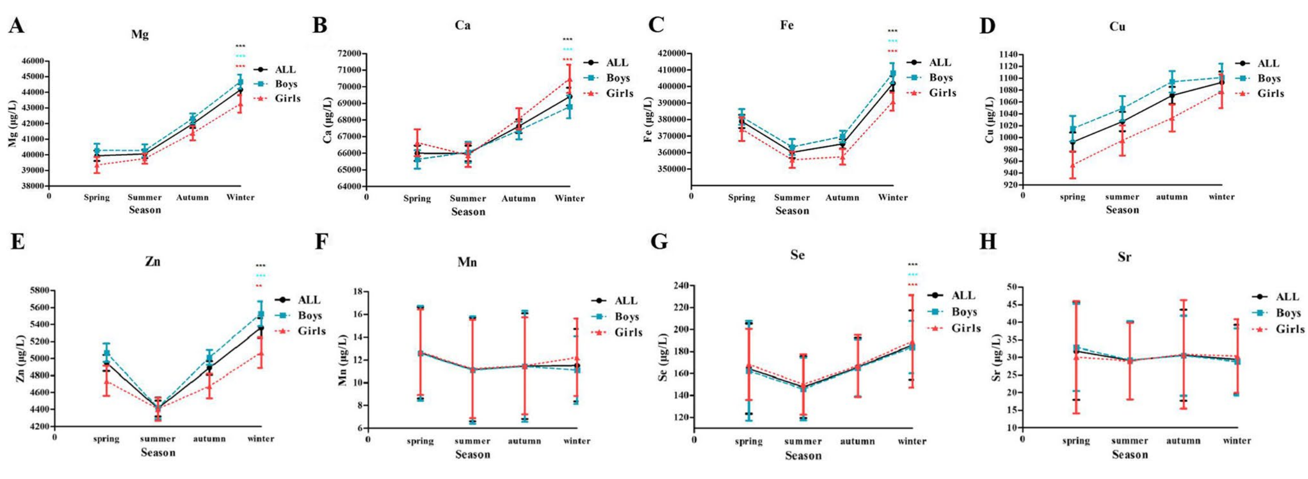

Fig. 2 The change trend of each nutrient element (μg/L) in successive seasons. Line charts were used to visually display the changes in the contents of various elements in the peripheral whole blood of boys or (and) girls with season. Data were expressed as means ± standard deviation. Element (Mg, Ca, Fe, Zn, Se) contents were higher in winter than that in summer (Zn for girls: **$p < 0.01$; other elements for all, boys, girls: ***$p < 0.001$)

Table 5 Reference intervals for nutrient elements (μg/L) 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)	Current RI in lab
Mg	31,920–47,280[a]	34,320–49,920[b]	34,800–50,640[b]	34,800–50,640[b]	33,120–50,640[a]	34,320–50,880[b]	34,560–49,200[b]	27,120–50,160
Ca	64,000–83,200[a]	59,200–80,000[a]	56,800–76,000[a]	56,000–73,200[b]	49,200–90,800[b]	56,000–80,800[b]	56,000–78,800[a]	46,000–84,000
Fe	248,080–397,600[a]	292,320–464,800[b]	296,240–452,480[a]	315,840–466,480[b]	292,880–525,840[a]	292,320–474,320[b]	281,120–453,040[a]	274,960–530,320
Cu	554.24–1515.52[a]	786.56–1529.60[b]	814.08–1440.64[b]	648.32–1447.68[b]	627.20–1477.76[b]	700.16–1509.12[b]	641.92–1461.12[b]	403.20–1814.40
Zn	1129.05–5185.05[a]	2817.75–6596.85[b]	3400.80–6652.10[a]	3785.60–6938.10[a]	3965–8092.50[a]	2669.55–7253.35[a]	2352.35–6999.85[a]	0–15: 3076.45–6748.30 ≥ 16: 4267.25–8236.80
Mn	1.76–17.32[a]	3.68–22.66[b]	4.50–19.16[b]	4.03–20.24[b]	3.64–31.19[b]	3.84–22.19[b]	4.02–20.16[b]	3–36
Se	69.11–195.49[a]	104.69–211.91[a]	113.29–224.73[b]	111.73–239.74[b]	103.46–234.94[a]	98.26–218.83[b]	93.19–236.41[b]	100–340
Sr	9.10–81.23[b]	14.11–56.18 [b]	13.81–54.13[b]	16.21–56.56[b]	10.82–57.74[a]	13.74–55.55[b]	14.51–59.29[b]	10–45

a: $\bar{x} \pm 1.96s$ for normally distributed data; b: $P_{2.5} \sim P_{97.5}$ for non-normally distributed data

It is necessary for laboratories to set recommended levels based on nutritional status and requirements.

In recent years, most studies reported on serum or plasma element levels in adult. Norway's national user manual in medical biochemistry recommend 63–126 μg/L of Se in the plasma [26]. The Swedish reference range is even lower at 55–95 μg/L [26]. Pacific BioLabs in USA reported that the levels of strontium in human serum samples range from 19 to 96 ng/mL [25]. Because of the influence of intracellular elements, the content of some elements in serum or plasma differs greatly from that in whole blood. Reports on the contents of elements in children and adolescents are more common in the hair [13, 27]. Assessing the contents of elements in the hair to evaluate the metabolic status in the individual is controversial because of environmental exposure to contaminants. Above all, the whole blood element level is a more valuable reflection of the element content in the body.

Diagnosis of trace element deficiency is really difficult. Appropriate nutritional deficiency threshold is dependent on age, gender, baseline nutritional history, and overall clinical status. Aschner M et al. reported that low concentrations of Mn in children (< 8.154 μg/L) have also been associated with lower color scores in the Stroop Color-Word Test [23]. The above cut-off-point for Mn is significantly higher than the lower limit of our established reference interval. But the measure reflects more cognitive flexibility and processing speed rather than nutritional status. Besides, we have not found other suggested cut-off-point for whole blood nutritional deficiencies of elements. But 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 [28]. 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 [29]. In this study, we evaluated a population of healthy children and adolescents, and the obtained data is hoped to be helpful for clinical assessment for determining the appropriate level of nutritional intake and the need for supplements in children and adolescents.

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, and 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 are 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

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Author Contribution FL and YW conceived and designed the study. SS, YC, YX, XJ, XG, and SC contributed to the 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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Data Availability The datasets used and analyzed in this study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval 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 to Participate Not applicable.

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

Competing Interests The authors declare no competing interests.

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