Seasonal Variation of Blood Calcium Levels in Children Aged 1–10

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Background: In this study, the associations of seasons with blood calcium levels in children aged 1–10 have not been evaluated. **Methods:** In 2012–2014, whole blood samples were collected from 2,562 children and calcium concentrations were determined by flame atomic absorption spectrometry. The associations of seasons with calcium levels were analyzed by multivariable regression. **Results:** The mean value of calcium concentrations was 1.61 ± 0.13 mmol/l and the overall deficiency was 29.3%. Overall, compared to those in winter, children in spring and summer had significant lower calcium concentrations that decreased by 1.2% ($\beta = -0.012$; 95% CI: $-0.021, -0.002$) and 1.4% ($\beta = -0.014$; 95% CI: $-0.023, -0.005$), re-

spectively; and corresponding higher calcium deficiencies than those in spring, summer, and autumn with odds ratios (OR) were 1.93 (95% CI: 1.39, 2.66), 1.65 (95% CI: 1.21, 2.24), and 1.57 (95% CI: 1.14, 2.15), respectively. Moreover, this seasonality was more significant in girls in whom calcium concentration in summer decreased by 1.9% ($\beta = -0.019$; 95% CI: $-0.036, -0.003$) and OR for calcium deficiencies in summer was 2.46 (1.38–4.41), compared to the girls in winter. **Conclusions:** The seasons have significant association with blood calcium levels, especially in girls. However, the impact of this seasonality on children's health is still unknown. J. Clin. Lab. Anal. 30:741–744, 2016. © 2016 Wiley Periodicals, Inc.

Key words: blood; calcium; children; odds ratios (OR); seasonal variation

Calcium is an essential element for human health and the fifth most abundant element in the human body. Only about 1% of total calcium of human body is present in blood, extracellular fluid, and other tissues. A very small amount of this immediately accessible calcium plays a key role in many biological functions such as extra- and intracellular signaling, membrane integrity, muscle contraction, neuronal transmission, hormonal secretion, and blood clotting (1–3).

The correlations between serum calcium levels and diseases have been previously studied. In adults, high serum calcium concentrations were highly correlated with cardiovascular disease and related death, even within the

normal range (4–6). In adolescents, high serum calcium concentrations were highly associated with hypertension (7). In addition, it is well known that insufficient calcium intake could have detrimental impact on bone health. Therefore, the variations in serum calcium concentrations of children and its influencing factors deserve our attention, not only for bone health but also for other health problems.

A previous study has shown that, in children, seasons of blood samples collection could have significant impact on serum calcium concentrations (8). However, this impact was only analyzed among children aged 11–18 and only compared for spring and autumn. Hence, the studies of seasonal variation of blood calcium concentrations in children are still lacking, especially in toddlers and preschool children. In addition, it is still unknown if seasons could have significant impact on blood calcium deficiencies.

To explore the relationship between season of blood samples collection, and calcium concentrations and its deficiencies, 2,562 relatively healthy children were recruited,

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including 1,719 boys and 843 girls aged 1–10. They all came for the routine health examination between January 2012 and December 2014 at the outpatient department of the Lu'an Affiliated Hospital of Anhui Medical University. Children on mineral supplements or vitamins were excluded from the study. Informed consent was taken from parents or guardians to use detection results and personal information of these children in this study. This study was approved by the Ethics committee of Lu'an People's Hospital. Seasons were defined as follows: spring (from March to May), summer (from June to August), autumn (from September to November), and winter (from December to February).

For all the children, after skin cleaning, about 2 ml of venous blood was drawn into vacuum tube (CHGD[®], Gaode, Wenzhou, China) with heparin sodium special for trace elements analyses. Whole blood concentrations of calcium, iron, copper, magnesium, and zinc were determined by flame atomic absorption spectrometry (BH5100, Bohui, Beijing, China) with hollow cathode lamps below 422.7, 248.3, 324.7, 285.2, and 213.9 nm, respectively. Quality controls were assured by analyzing certified reference materials GBW(E)080915, 080916, and 080917 from the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China. Reference samples were analyzed after every ten routine sample detection. If the values exceeded the uncertainty provided by the certificate of reference materials, the previous ten routine samples must be reanalyzed. Reference value for calcium was 1.55–2.65 mmol/l.

Data analysis was performed using SPSS version 19.0 statistical software. The associations of natural log-transformed calcium concentrations and calcium deficiencies (<1.55 mmol/l) with seasons were determined using multivariable linear and logistic regression, respectively. In multivariable linear regression, the blood levels of iron, copper, magnesium, and zinc were natural log-transformed. The changes in blood calcium levels in multivariable linear regression were calculated as $(1 - e^{\beta}) \times 100\%$. A *P*-value <0.05 (two-tailed) was considered as statistically significant.

The overall mean value (mean $\pm$ SD, together with median range) of calcium concentrations was 1.61 ± 0.13 mmol/l (1.60, 1.20–2.12) and the overall deficiency was 29.3%. In multivariable regression, after controlling for sex, age, year of samples collection, and concentrations of blood iron, copper, magnesium, and zinc, season was significantly associated with both blood calcium concentrations and its deficiencies (Table 1).

The blood calcium concentrations of children in spring and summer decreased by 1.2% ($\beta = -0.012$; 95% CI: $-0.021, -0.002$; *P* = 0.02) and 1.4% ($\beta = -0.014$; 95% CI: $-0.023, -0.005$; *P* = 0.003), respectively, compared to the concentrations in winter. Children in spring, summer,

and autumn had 1.93 (OR = 1.93; 95% CI: 1.39, 2.66; *P* < 0.001), 1.65 (OR = 1.65; 95% CI: 1.21, 2.24; *P* = 0.001), and 1.57 (OR = 1.57; 95% CI: 1.14, 2.15; *P* = 0.005) times the risk of calcium deficiencies, respectively, compared to the children in winter. The blood calcium concentrations of girls in spring and summer decreased by 1.8% ($\beta = -0.018$; 95% CI: $-0.036, -0.001$; *P* = 0.04) and 1.9% ($\beta = -0.019$; 95% CI: $-0.036, -0.003$; *P* = 0.02), respectively, compared to the girls in winter. The girls in spring, summer, and autumn had 2.28 (OR = 2.28; 95% CI: 1.23, 4.25; *P* = 0.009), 2.46 (OR = 2.46; 95% CI: 1.38, 4.41; *P* = 0.002), and 1.91 (OR = 1.91; 95% CI: 1.05, 3.48; *P* = 0.03) times the risk of calcium deficiencies, respectively, compared to the girls in winter. Only the boys in summer had significantly decreased calcium concentrations ($\beta = -0.011$; 95% CI: $-0.023, -0.001$; *P* = 0.04) compared to the boys in winter, and only the boys in spring had significantly higher risk of calcium deficiencies (OR = 1.77; 95% CI: 1.20, 2.59; *P* = 0.004) compared to the boys in winter. These results showed that seasons were significantly associated with both calcium levels, and these associations were more significant in girls than in boys.

Dietary calcium is primarily absorbed by the small intestine, and this is a function of active transport controlled by 1,25(OH)₂D that is derived from cutaneous synthesis of vitamin D during exposure to ultraviolet B radiation (UVB) light (3). It is conceivable that serum 1,25(OH)₂D levels are the highest in summer than in other seasons (9, 10). More serum levels of 1,25(OH)₂D could promote more calcium absorption and ensue more blood calcium concentrations. Therefore, the serum calcium concentrations in people in summer are theoretically higher than in winter. However, our results showed that the blood calcium concentrations of Lu'an children in summer were significantly lower than the children in winter, and correspondingly the calcium deficiencies in summer was significantly higher than the children in winter (Table 1). These results indicated that factors other than serum 1,25(OH)₂D levels, such as the calcium contents of various kinds of food, may also play important roles in determining blood calcium levels.

Chinese Spring Festival takes place in winter, and people usually consume many calcium-rich foods such as soybean, bean curd, and other soy products; pork soup; and fishes during those days and the ensuing month. Furthermore, during wintertime, people here are prone to consume more powdered milk instead of liquid milk, and soymilk powder and oats that need to be brewed by boiling water in winter than in other seasons. These special dietary habits during wintertime might be an important reason for higher blood calcium concentrations in winter than in summer in children from Lu'an. However, more studies are needed to carefully investigate the dietary structure of children in

TABLE 1. Association of Seasons With Blood Calcium Levels Based on Multiple Regression

	Linear regression ^a			Logistic regression ^b		
	β	95% CI	P-value	OR	95% CI	P-value
Total						
Winter		Reference			Reference	
Spring	−0.012	−0.021 to −0.002	0.02	1.93	1.39–2.66	<0.001
Summer	−0.014	−0.023 to −0.005	0.003	1.65	1.21–2.24	0.001
Autumn	−0.006	−0.016 to −0.003	0.20	1.57	1.14–2.15	0.005
Boys						
Winter		Reference			Reference	
Spring	−0.009	−0.020 to 0.003	0.14	1.77	1.20–2.59	0.004
Summer	−0.011	−0.023 to −0.001	0.04	1.36	0.94–1.96	0.11
Autumn	−0.004	−0.016 to 0.008	0.49	1.44	0.99–2.09	0.06
Girls						
Winter		Reference			Reference	
Spring	−0.018	−0.036 to −0.001	0.04	2.28	1.23–4.25	0.009
Summer	−0.019	−0.036 to −0.003	0.02	2.46	1.38–4.41	0.002
Autumn	−0.011	−0.028 to 0.006	0.21	1.91	1.05–3.48	0.03

^aDependent variable was ln-transformed blood calcium concentration and model was adjusted for age, sex, year, ln-Fe, ln-Cu, ln-Mg, and ln-Zn.

^bDependent variable was children with calcium concentrations <1.55 mmol/l and model was adjusted for age, sex, year, Fe (mmol/l), Cu (μ mol/l), Mg (mmol/l), and Zn (μ mol/l).

different seasons, and its relationship with blood calcium levels.

Lead could compete with calcium for binding sites of intestinal mucosal proteins and therefore inhibit calcium uptake (11). Studies previously reported showed that blood lead levels had significant seasonal variations with the highest value in summer, which was significantly higher than in winter (12, 13). Therefore, seasonal variations of lead absorption may cause seasonal variations of calcium uptake and blood calcium concentrations. However, our previous study demonstrated that in Lu'an children, blood lead concentrations were highest in winter (14), and this is consistent with seasonal variation of blood calcium concentrations in this study. Therefore, the seasonal variations of blood calcium levels of children from Lu'an might not be an effect of lead in the environment.

There were some infectious diseases that demonstrate seasonal prevalence in children, such as the rotavirus infection, respiratory syncytial virus, and influenza virus (15–17). Few studies also show that extracellular calcium has important effects on the function of monocytes and dendritic cells (18, 19). Furthermore, other studies show seasonal variation in peripheral blood human T-cell numbers and their phenotypes, which was associated with seasonal variation in serum 1,25(OH)₂D levels (9). Dietary calcium is primarily absorbed by the small intestine controlled by 1,25(OH)₂D (3). Therefore, seasonal variation in peripheral blood human T cells might also be associated with seasonal variation of blood calcium levels. All these results suggest that there might be some correlations between seasonal prevalence of infectious diseases and seasonal variation of blood calcium levels in children.

In summary, the seasonal variation of blood calcium levels in children from Lu'an suggests that the importance of sufficient calcium intake is different between seasons, especially in girls, and when calcium supplementation is carried out in children, the seasonality should be taken into consideration for maintaining stable blood calcium concentrations across four seasons. In future studies on the relationships between blood calcium levels and diseases, the seasonality of calcium levels should be included as a confounding factor.

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CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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