

# Relationships Between Dietary Patterns And Low Level Lead Exposure Among Children From Hunan Province of China

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## Research Article

**Keywords:** Dietary patterns, Blood, Lead, Children

**Posted Date:** July 21st, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-656647/v1>

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# Abstract

**Background:** Lead (Pb) is a potent environmental toxic metal. Few studies have focused on low-level Pb exposure in children. This study evaluated the relationships of dietary patterns with low-level Pb exposure in children from Hunan province of China.

**Methods:** A cross-sectional study was conducted in a single primary school, located in Hunan Province. In total, 425 children were recruited. Principal component analysis was used to identify dietary patterns based on dietary intake from a food frequency questionnaire (FFQ). The blood lead level (BLLs) was measured. Multivariate regression analysis was used to investigate the correlations of BLLs with dietary patterns.

**Results:** Three dietary patterns were identified: balanced, plant, and beverage and snack. The concentration of blood lead (median (IQR)) was 2 (2, 15.96)  $\mu\text{g/L}$ . Only 0.24% children's BLLs were  $>100 \mu\text{g/L}$  and 1.18% children's BLLs were  $>50 \mu\text{g/L}$ . No significant risk association was found between dietary patterns and BLLs by logistic regression analysis based on 50th percentile (P50) of blood lead. However, children's gender of girl decreased the risk of BLLs (AOR=0.57, 95% CI: 0.38, 0.85, P=0.006). The plant pattern had weekly positive association with log-Pb (B=0.04, 95% CI: 0.00, 0.08, P=0.035) in group of  $> P50$  by linear regression analysis.

**Conclusions:** BLLs was low among children aged 4–7 years in Hunan province of China. Low BLLs had no association with dietary pattern, mediated by some confounding factors, such as gender. However, diet may become an important source of blood lead with increasing blood lead levels.

## Introduction

Lead (Pb) is a potent environmental toxic metal that contributes to 0.6% of the disease burden worldwide (World Health Organization 2010). Children are more vulnerable to Pb exposure. Blood lead levels (BLLs) of  $< 100 \mu\text{g/L}$  and  $< 50 \mu\text{g/L}$  are considered safe for children according to the World Health Organization and United States Environmental Protection Agency, respectively (Zhang, et al. 2020).

Many studies have shown that high-level Pb exposure is associated with adverse health effects on the developing central nervous system in children (Desrochers-Couture, et al. 2018, Huang, et al. 2016, World Health Organization 2010). Other studies have reported that exposure to high-level Pb may lead to elevated blood pressure (BP) in children, thereby increasing the risks of hypertension and cardiovascular diseases in adulthood (Chen and Wang 2008). However, few studies have focused on the associations between health effects and low-level Pb exposure in children. Several studies have suggested there is no suitable threshold BLLs, and BLLs  $< 50 \mu\text{g/L}$  may be associated with adverse health effects in children (Earl, et al. 2016, Skerfving, et al. 2015, Zhang, et al. 2020). Thus, there has been increasing attention regarding health outcomes associated with low-level Pb exposure in children.

The various sources of human exposure to Pb include contaminated food, drinking water, air, and dust, as well as industrial activities (Gao, et al. 2020, Gibson, et al. 2020, Heusinkveld, et al. 2021, Safruk, et al. 2017, Woo, et al. 2018). Diet is the principal source of individual Pb exposure; it contributes more than 80% of overall Pb intake (Li, et al. 2016). Children are more susceptible to dietary Pb exposure because they require more food and water, compared with adults, relative to their body weight (Gao, et al. 2020). However, most studies regarding dietary exposure to Pb in children have mainly focused on a single food (Bjermo, et al. 2013, Gao, et al. 2020, Tamayo y Ortiz, et al. 2016). These studies have not evaluated dietary sources of Pb from combinations of diverse foods and nutrients because some dietary nutrients (e.g., iron) can interfere with the intestinal absorption of Pb (Shah-Kulkarni, et al. 2016). Dietary pattern analysis has recently been developed to investigate comprehensive dietary links with health (Sanchez, et al. 2020, Shi 2020) to better represent nutritional exposures and reflect daily eating habits.

This cross-sectional study investigated blood samples collected among children aged 4–7 years, with the aim of evaluating the lead burden in children from Hunan province of China and identifying the influence of dietary patterns on BLLs.

## Materials And Methods

### *Study design and study population*

This cross-sectional study was conducted in Xiangtan, Hunan Province, located in South Central China. All children aged 4–7 years in a single primary school were invited to participate. In total, 435 children were recruited; 10 were excluded due to incomplete dietary assessment. Experimental strategy for linking dietary patterns and lead exposure in children was shown in Fig. 1.

### *Dietary assessment and food grouping*

Dietary intake information was collected using a validated food frequency questionnaire (FFQ) to evaluate children's dietary intake (Chinese Center for Disease Control and Prevention 2015). Caregivers were asked to recall each child's intake (including estimated portion size and frequency of each food item) over the previous 12 months. The frequency was recorded in terms of times per day, week, month, or year; portion sizes were expressed in grams or milliliters. The mean daily intake of each food item was calculated using the estimated portion size and frequency. Total of 55 food items were further categorized into 19 food groups based on similarities in nutrient profiles or processing methods. These foods included rice, wheat flour, coarse cereals, soybeans and their products, meat, poultry, eggs, fish, shrimp, crab and shellfish, leafy vegetables, leafless vegetables, tubers, fresh beans, fungi and algae, fruits, milk and its products, nuts, beverages, and snacks. The FFQ was administered by a well-trained dietitian.

Principal component analysis was used to identify dietary patterns, as in our previous study (Huang, et al. 2019). The main dietary patterns were identified based on eigenvalue scree plots, factor interpretability, and variance explained. Factor loadings represent correlation coefficients between food items and dietary

patterns; factor loadings > |0.3| were regarded as primary contributors. Factor scores (i.e., summed intakes of each food group weighted according to factor loading) were calculated for each pattern and each individual. Factor scores were categorized into four quartiles, such that Q1 was weakly related to the dietary pattern and Q4 was strongly related to the dietary pattern.

### *Lead intake*

Lead intake of each child was estimated based on the food intake in this study and food lead concentration data from previous study (Jin, et al. 2014). The lead concentration (mean lead in each food category) was based on lead measurements in 2077 food samples from 23 food categories during 2007–2010(Supplement 1).

### *Blood sampling and analysis*

Peripheral venous blood samples (3mL/child) were drawn from the cubital vein using a vacuum tube with an anticoagulant. BLLs were measured using graphite furnace atomic absorption spectrometry (ContrAA 700; Analytik Jena GmbH, Jena, Germany). Recorded values were the means of triplicate sample analyses. The limit of detection for Pb was 4.0µg/L. The value below the limit of detection was calculated as half of the detection limit.

### *Assessment of growth and development*

Weight (kg) and height (cm) were measured with an electronic height and weight measurement instrument. Body Mass Index (BMI) was calculated by weight (kg) dividing square of height (m). An appropriate cuff size and a mercury sphygmomanometer were used to measure BP. Systolic blood pressure (SBP) and diastolic blood pressure (DBP), expressed in mmHg, were recorded from two consecutive BP measurements. Cognitive performance was assessed using the Clinical Memory Scale Test, developed by the Institute of Psychology of the Chinese Academy of Science(Xu,SL., et al. 1996). This test was mainly used to evaluate short-term memory performance, including directed memory, associative memory, free image recall, meaningless image recognition, and associative image memory. First, each section was scored according to the results of a test for each student. Then, the score was changed to a scale and the total value was determined. Last, the total scale score was converted into a memory quotient (MQ) according to age group, which reflected comprehensive memory performance.

### *Other related variables*

Information regarding demographic characteristics was collected during the FFQ interview. These variables included age, sex, caregiver group (parents or grandparents/others), caregiver occupation (public institution, non-public institution, or unemployed), caregiver education (college and above, senior, or junior and below), and annual family economic level ( $\geq$  50,000 yuan, 20,000–50,000 yuan, or < 20,000 yuan). Age was divided into two groups (4–5 years or 6–7 years).

### *Ethics approval and consent to participate*

All participants provided written informed consent. The study protocol was approved by the Ethics Committee of the Hunan Provincial Center for Disease Control and Prevention.

### *Statistical analysis*

Continuous variables were expressed as mean and standard deviation (SD) for normal distribution or medians and inter-quartile ranges (IQR) for Skewed distribution. Categorical variables were expressed as numbers and percentages. BLLs were categorized into two groups according 50th percentile (P50). The demographic characteristics were compared according to BLLs, using analysis of Variance (ANOVA) or chi-square test. Correlation of factor scores with lead intake was assessed using Pearson correlation analysis. Logistic regression analysis was used to investigate the correlations of BLLs with dietary patterns. Next, log-Pb was calculated in children in group of > P50. The associations between log-Pb and dietary pattern were tested in linear regression analysis.  $P < 0.05$  was considered to indicate statistical significance. Statistical analyses were performed using SPSS software (version 13.0; SPSS, Inc., Chicago, IL, USA).

## **Results**

### *Participant dietary patterns*

Dietary pattern analysis revealed three dietary patterns among children in this study. The factor loadings of each pattern are shown in Fig. 2. Pattern 1 was regarded as the balanced pattern: high intakes of fruits, nuts, leafless vegetables, poultry, fungi and algae, fresh beans, tubers, fish, meat, soybeans and their products, snacks, rice, and shrimp, and crab and shell fish. Pattern 2 was regarded as the plant pattern: high intakes of coarse cereals, soybeans and their products, leafless vegetables, tubers, and wheat flour, with lower intakes of poultry and meat. Pattern 3 was labeled as the beverage and snack pattern: high intakes of beverages, snacks, and milk and its products, with low intakes of shrimp, crab and shellfish, and fish. In addition, compared with the lowest quartile, the intakes of 19 food groups were higher in the highest quartile of the balanced pattern. The intakes of eggs, fish, milk and its products, leafy vegetables, nuts, and snacks were lower in the highest quartile of the plant pattern. Beverage and snack patterns had lower intakes of eggs, leafless vegetables, and fungi and algae in the highest quartile (Supplement 2). The association of dietary patterns and lead intake was shown in Fig. 3. The balance pattern ( $r = 0.841$ ,  $P < 0.001$ ) and beverage & snack pattern ( $r = 0.315$ ,  $P < 0.001$ ) had positively associated with lead intake.

### *Distribution of BLLs in Hunan children*

The concentration of blood lead (median (IQR)) was 2 (2, 15.96)  $\mu\text{g/L}$ . Figure 4 presented the frequency distribution of BLLs in Hunan children. More than half (50.82%) of the children had BLLs below the limit of detection. Only 0.24% children's BLLs were  $> 100 \mu\text{g/L}$  and 1.18% children's BLLs were  $> 50 \mu\text{g/L}$ . BLLs were categorized into two groups ( $\leq P50$  or  $>P50$ ) according 50th percentile of blood lead. Table 1 displayed the demographic characteristics of children in different groups of BLLs. Children with group of  $>P50$  had higher proportion of boys (59.81%) than group of  $\leq P50$  (46.76%) ( $P = 0.007$ ). There was no

significant difference for age, caregiver group, caregiver occupation, caregiver education, and annual family economic level in different groups of BLLs. In addition, no significant correlations were found between BLLs and BMI, blood pressure or cognitive development.

Table 1 Sample characteristics of Hunan children by 50th percentile of blood lead

	≤P50 (n=216)		≥P50 (n=209)		P
Gender (%)					
Boy	101	(46.76)	125	(59.81)	0.007
Girl	115	(53.24)	84	(40.19)	
Age (%)					
4 to 5 year	107	(49.54)	105	(50.24)	0.885
6 to 7 year	109	(50.46)	104	(49.76)	
Caregiver (%)					
Parents	128	(59.26)	119	(56.94)	0.682
Grandparents and others	88	(40.74)	90	(43.06)	
Caregiver's occupation (%)					
Public institution staff	21	(9.72)	18	(8.61)	0.924
Non public institution staff	77	(35.65)	75	(35.89)	
Unemployment	118	(54.63)	116	(55.50)	
Caregiver's education (%)					
College and above	20	(9.26)	18	(8.61)	0.078
Senior	71	(32.87)	49	(23.44)	
Junior and below	125	(57.87)	142	(67.94)	
Family economic level (%)					
50,000 and above	69	(31.94)	63	(30.14)	0.885
20,000 to 50,000	96	(44.44)	93	(44.50)	
Below 20,000	51	(23.61)	53	(25.36)	
BMI	15.08	1.77	15.06	1.77	0.922
Blood pressure (mmHg)					
SBP	91.85	10.50	93.01	9.60	0.235
DBP	58.85	9.43	59.24	8.14	0.650
Memory quotient	54.72	12.39	55.31	11.66	0.650

Values are means  $\pm$  SD unless otherwise indicated; P values were calculated by using ANOVA or chi-square test

*Relationships of dietary patterns with BLLs*

Table 2 indicated that comparing to group of  $\leq$  P50, no increasing lead intake was found in group of >P50. No significant risk association was found between dietary patterns and BLLs. However, children's gender of girl decreased the risk of BLLs (AOR = 0.57, 95%CI: 0.38, 0.85, P = 0.006), after adjusting for age, caregiver's groups, caregiver's occupation, education, family economic level, dietary patterns and lead intake.

Table 2 The association of demographic characteristics and diet with blood lead ( $\leq$ P50 vs  $>$ P50) among Hunan children by logistic regression analysis (N=425)

Diet	COR (95.0% CI)	P	AOR (95%CI)	P
Lead intake	1.00 (0.99,1.01)	0.927	1.00 (0.99,1.01)	0.999
Balance pattern				
Q1	1.00		1.000	
Q2	1.16 (0.679,1.99)	0.583	1.09 (0.63,1.90)	0.755
Q3	0.95 (0.55,1.62)	0.840	0.86 (0.49,1.51)	0.594
Q4	1.08 (0.63,1.85)	0.784	1.04 (0.59,1.86)	0.888
Plant pattern				
Q1	1.00		1.00	
Q2	1.49 (0.87,2.55)	0.150	1.50 (0.86,2.62)	0.151
Q3	1.41(0.82,2.42)	0.216	1.48 (0.85, 2.61)	0.169
Q4	1.21 (0.70,2.08)	0.491	1.29(0.74,2.25)	0.376
Beverage & snack pattern				
Q1	1.00		1.00	
Q2	0.77 (0.45,1.32)	0.336	0.83 (0.47,1.46)	0.514
Q3	1.06 (0.62,1.81)	0.838	1.09 (0.62,1.91)	0.766
Q4	1.08 (0.63,1.85)	0.784	1.14 (0.65,1.98)	0.653
Gender				
Boy	1.00		1.00	
Girl*	0.59 (0.40,0.87)	0.007	0.57 (0.38,0.85)	0.006

COR : Crude odds ratios, AOR :adjusted odds ratios , adjusted for age, gender, caregiver's groups, caregiver's occupation, education, family economic level.\* AOR : adjusted for age, caregiver's groups, caregiver's occupation, education, family economic level, dietary patterns and lead intake.

In addition, log-Pb was calculated in children with group of >P50. The correlation of 19 food groups and lead intake with BLLs was conducted by Pearson correlation analysis. The result showed that meat intake had negative association with log-Pb, but fruit intake had positive association with log-Pb (Supplement 3). The associations between log-Pb and dietary pattern were tested in linear regression analysis. The result showed that plant pattern had weekly positive association with log-Pb (B = 0.04, 95% CI: 0.00, 0.08, P = 0.035), after adjusting for age, gender, caregiver's groups, caregiver's occupation, education, family economic level. No significant association was found between BLLs and lead intake, balance plant or beverage & snack pattern (Fig. 5).

## Discussion

This cross-sectional study examined the relationships of Pb exposure with dietary patterns in children from Hunan province of China. There were four key findings. First, three dietary patterns were identified: balanced, plant, and beverage & snack. The balance pattern and beverage & snack pattern had positively associated with lead intake. Second, only 0.24% children's BLLs were > 100µg/L and 1.18% children's BLLs were > 50 µg/L. BLLs was low among children from Hunan province. Third, no significant risk association was found between dietary patterns and BLLs in children with low-level Pb exposure, but children's gender of girl decreased the risk of BLL. Fourth, a weekly positive association was found between plant dietary pattern and BLLs in group of > P50.

In this study, we found that 50.82% of children had BLLs < 4 µg/L. Only 0.24% and 1.18% children had BLLs that exceeded current guidelines from the World Health Organization (100 µg/L) and the United States Environmental Protection Agency (50 µg/L), respectively(Zhang, et al. 2020). This finding suggests that the children in our study had low-level Pb exposure. Many studies have demonstrated low contemporary BLLs among children in China. A national cross-sectional study of children in China indicated that the median BLLs was 26.7 µg/L(Li, et al. 2020). Zhang et al.(Zhang, et al. 2020) reported a gradual decrease in BLLs among Chinese children from 182.9 µg/L(1987–1991) to 42.4 µg/L (2012–2017).

Food is an important source of Pb exposure. Most studies have focused on relationships of diet with Pb toxicity (Lacasana, et al. 2000, Schell, et al. 2004). In this study, three dietary patterns were identified. The balance contained a variety of foods, closely adhered to Chinese dietary guidelines(Yang, et al. 2017). The plant pattern had low intakes of poultry and meats. The intakes of milk and its products were far lower than recommended in dietary guidelines. The beverage and snack pattern (i.e., high intakes of beverages and snacks) may lead to health risks, such as obesity (Xu, et al. 2019). In this study, the balance pattern and beverage & snack pattern had positively associated with lead intake. Shi et al (Shi, et al. 2019) reported that iron-related dietary pattern was significantly positively associated with lead intake.

However, we found dietary patterns had no association with BLLs. Previous studies also have shown that a western food pattern is positively correlated with higher bone Pb concentrations (Wang, et al. 2017), while a balanced food pattern may reduce the risk of elevated BLLs in adults (Chung, et al. 2013). This result hinted that the association of diet and BLLs might be mediated by some confounding factors in low level lead exposure. In addition, we found BLLs had association with gender in low level lead exposure. Gender with girl decreased the risk of BLLs. Similar results were also reported by Lim et al (Lim, et al. 2015) and Zhang et al (Zhang, et al. 2020). The gender difference may be attributed to difference of sex on many factors, such as haematocrit levels (Becker, et al. 2002), outdoor activity time, personality traits, and health habits (Wang and Zhang 2006). This result suggested that low BLLs was impacted by many factors.

Moreover, the association of dietary patterns with blood lead was analyzed in group of > P50. We found that the plant dietary pattern was positively correlated with BLLs in children. However, lead intake had no association with plant pattern in this study. The reason may ascribe that overall dietary intake may modify the intestinal absorption of Pb due to interactions among specific foods or nutrients. Recent human studies reported that there were interactions between iron deficiency and lead poisoning. High iron intake and sufficient iron stores may reduce the risk of lead poisoning (Kwong, et al. 2004, Lacasana, et al. 2000, Schell, et al. 2004). Another study found that higher intake of milk and dairy rich in calcium was associated with reduced BLLs (Kordas, et al. 2018). In our study, the plant dietary pattern included high intakes of coarse cereals, soybeans and their products, leafless vegetables, tubers, and wheat flour. Those foods contain low levels of iron and calcium. Moreover, plant pattern included lower intakes of poultry and meats. Meat intake had negative association with log-Pb had been verified in this study. This result suggested that diet may become an important source of blood lead with increasing blood lead levels.

There were some strengths in this study. Notably, it explored low-level Pb exposure in children, which has been neglected previously. Furthermore, this study used dietary pattern analysis to assess the relationships between diet and BLLs, providing specific insights for nutritional intervention. However, the study also had several limitations. First, a causal relationship could not be established because of the cross-sectional design. Second, we used a FFQ method to collect dietary data, which may have been subject to recall bias. Lastly, the participants were selected from only one school, which may have affected the generalizability of the findings.

## **Conclusion**

This study showed that BLLs was low among children aged 4–7 years in Hunan province of China. Furthermore, low BLLs had no association with dietary pattern, mediated by some confounding factors, such as gender. However, diet might become an important source of blood lead with increasing blood lead levels. The findings provide insights for reducing the burden of low-level Pb exposure in children.

## **Abbreviations**

B: Unstandardized beta

BLLs: Blood lead levels

BP: Blood pressure

BMI: Body Mass Index

ANOVA: Analysis of Variance

CI: Confidence interval

CMS: Clinical memory scale test

DBP: Diastolic pressure

FFQ: Food frequency questionnaire

GFAAS: Graphite furnace atomic absorption spectrometry

IQR: Inter-quartile range

MQ: Memory quotient

OR: Odds ratio

Pb: Lead

PCA: Principal component analysis

P50: 50th percentile

SBP: Systolic pressure

SD: Standard deviation

## **Declarations**

### ***Acknowledgement***

We thank the participants for their assistance and support.

### ***Ethics approval and consent to participate***

All participants signed the informed consent form, and the study procedure was approved by the ethics committee of Hunan Center for Disease Control and Prevention.

## ***Conflict of Interest Statement***

No conflict of interest exists in the submission of this manuscript, and manuscript is approved by all authors for publication.

## ***Funding information***

Self-funded

## ***Authors' contributions***

ZH, BC, XC, MC and YH performed the research. ZH, BC and YH designed the research study. BC and YH contributed essential reagents or tools. ZH, MC and ML analysed the data. ZH wrote the paper.

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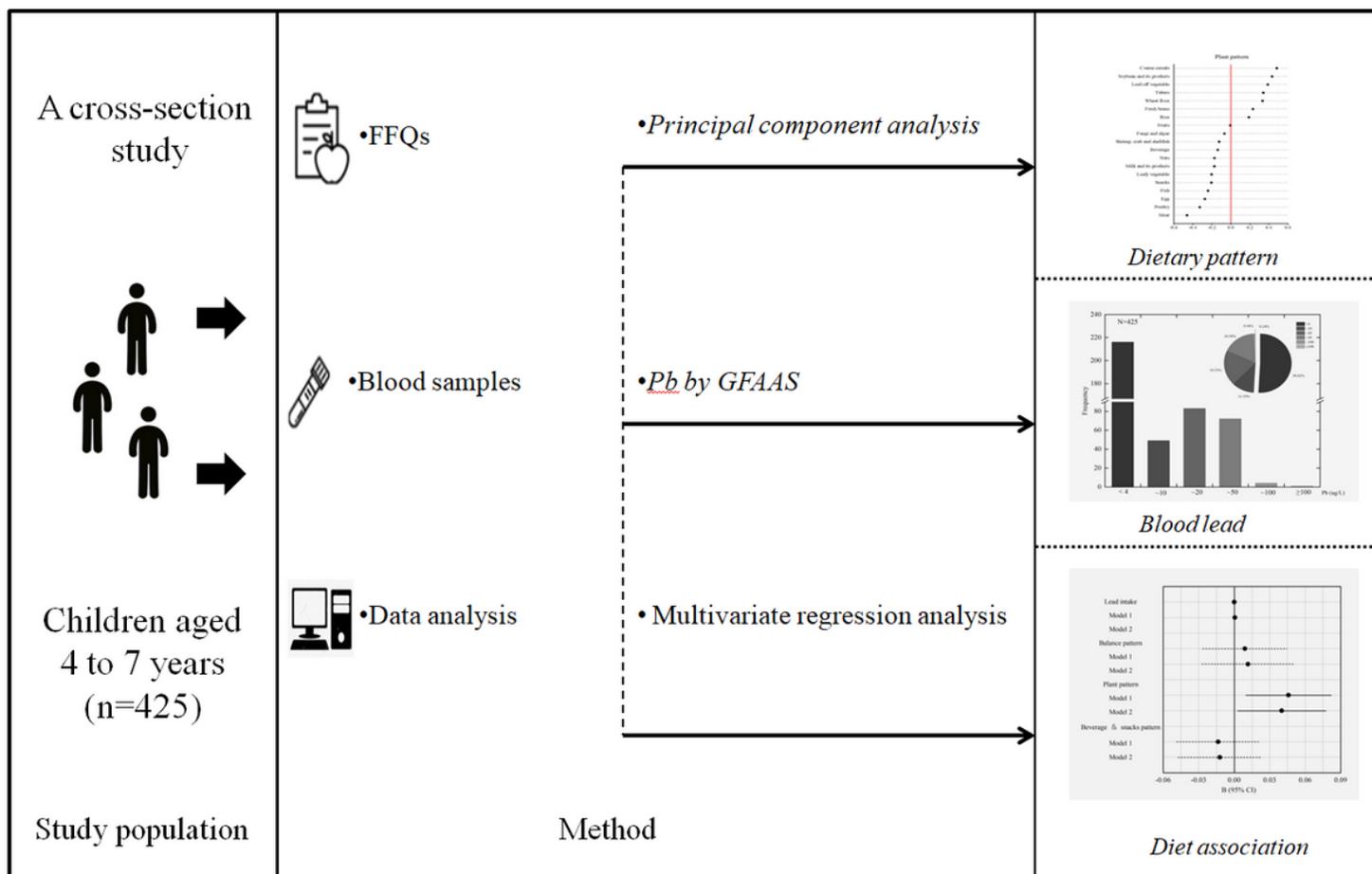
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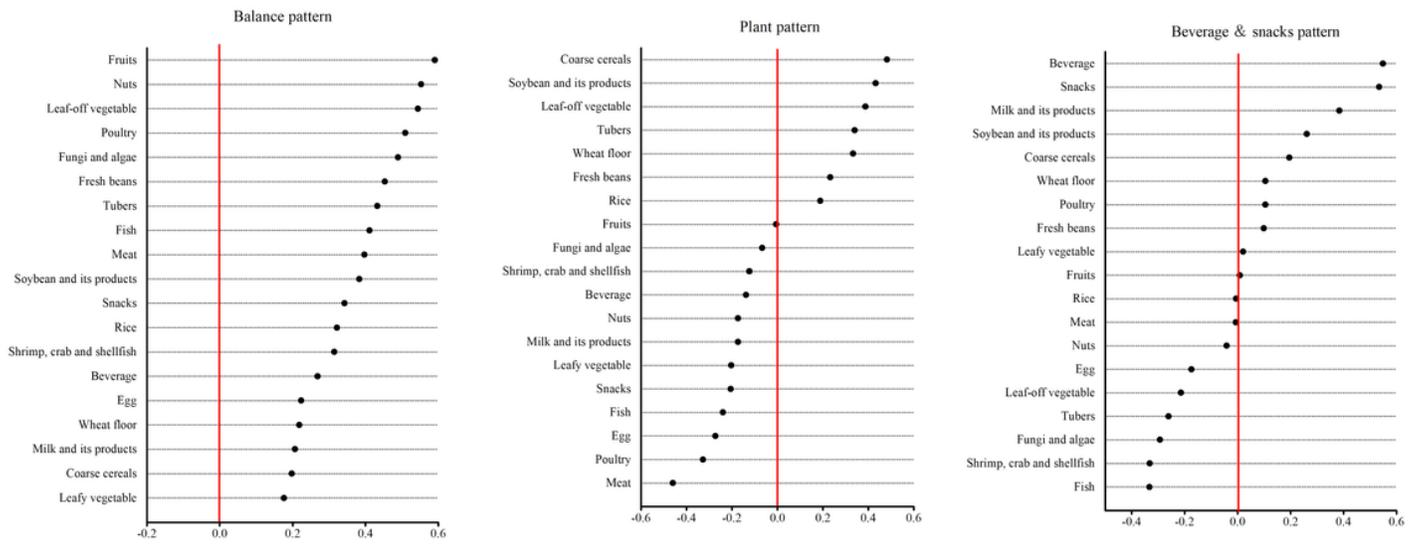
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## Figures



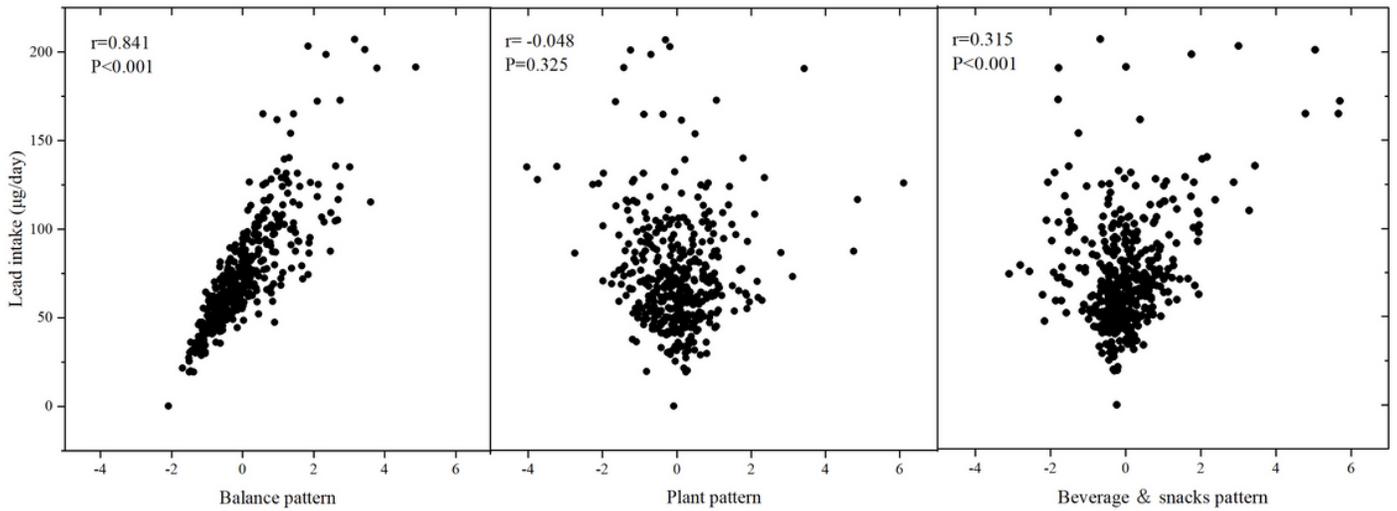
**Figure 1**

Experimental strategy for linking dietary patterns and lead exposure in children. FFQ: Food frequency questionnaire; GFAAS: graphite furnace atomic absorption spectrometry.



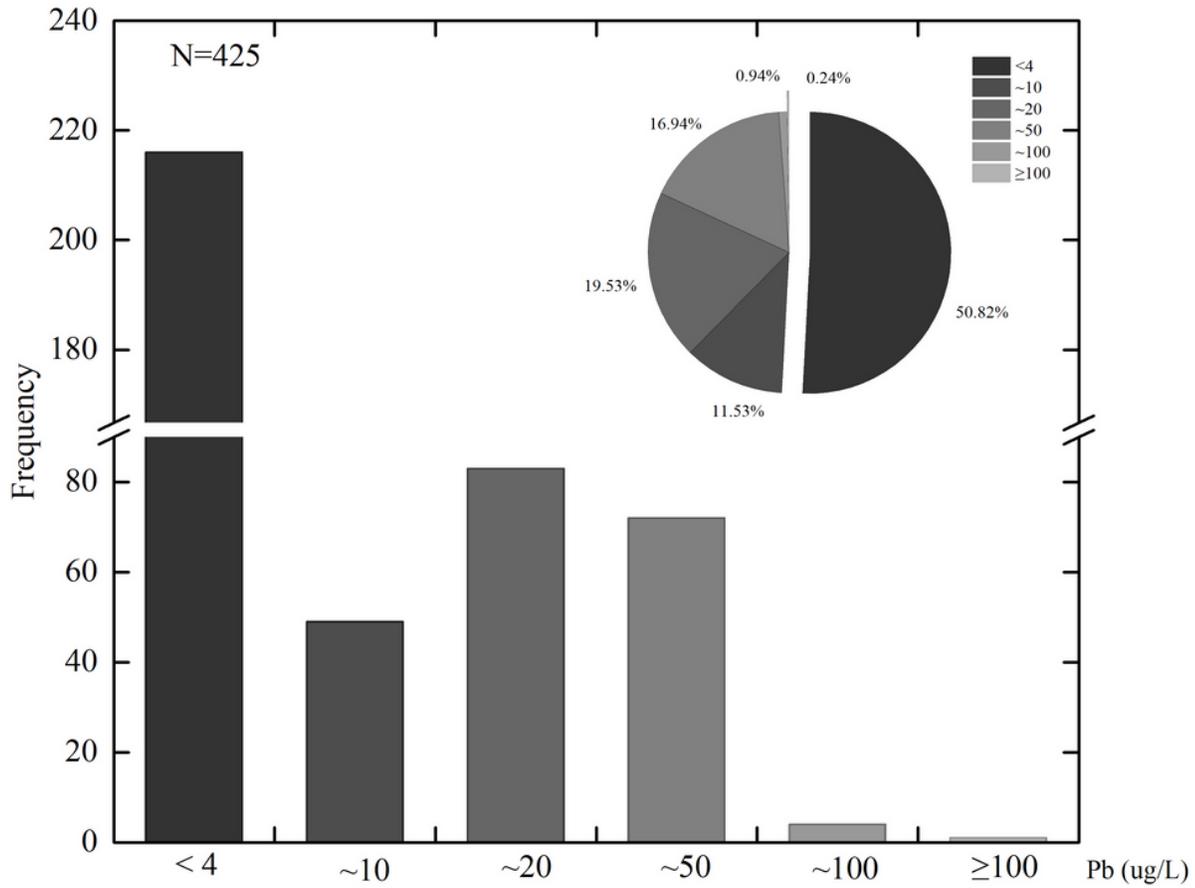
**Figure 2**

Factor loadings for dietary patterns of children. (a): Balance plant; (b): Plant pattern; (c): Beverage & snack pattern.



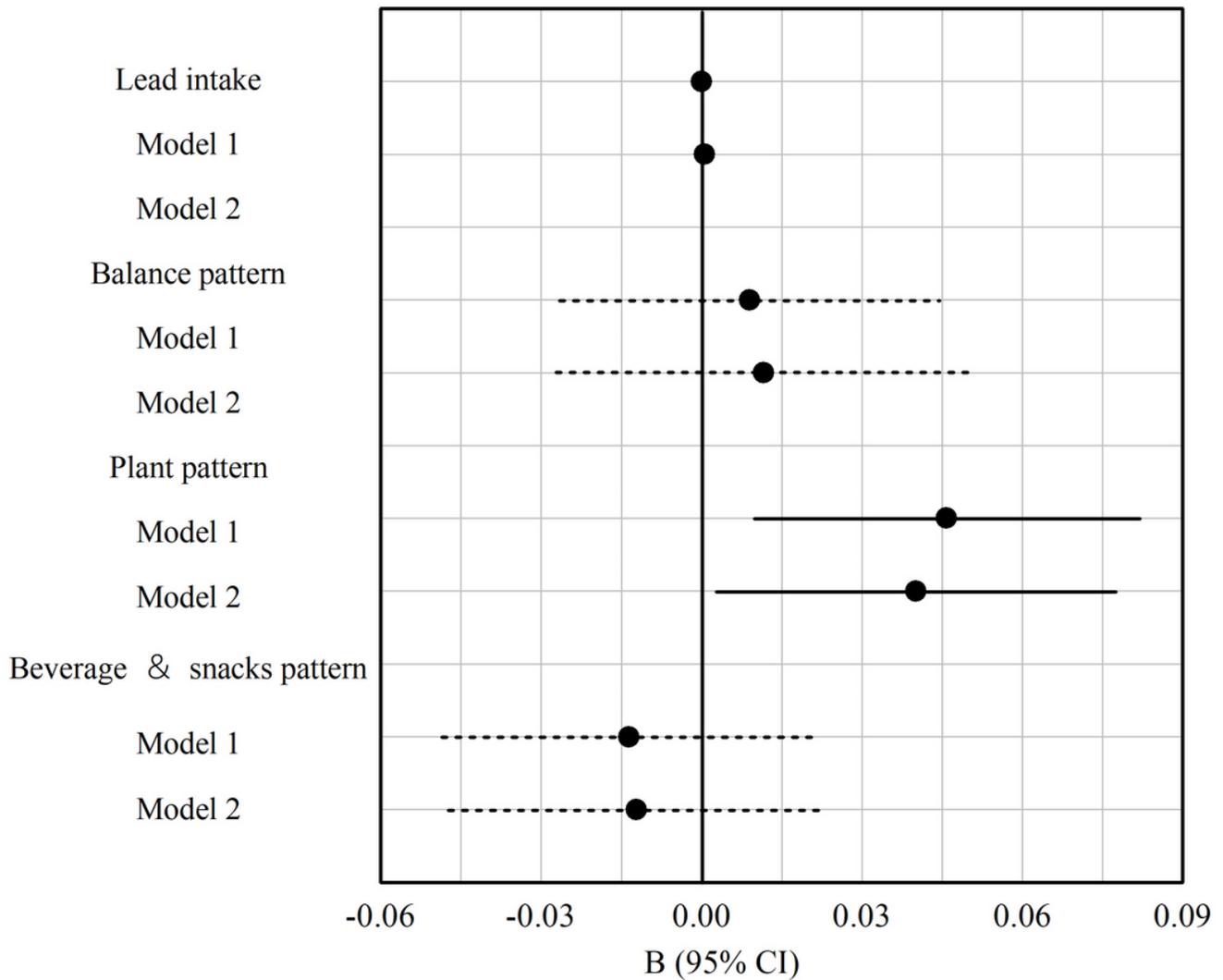
**Figure 3**

Correlation of dietary factor scores with lead intake by Pearson correlation analysis.



**Figure 4**

The chart of frequency distribution for blood Pb concentrations in Chinese children.



**Figure 5**

The relationship between dietary patterns and blood log-Pb among group of children with blood lead >P50 by linear regression analysis. Model 1 Unadjusted Model 2: Adjusted age, gender, caregiver's groups, caregiver's occupation, education, family economic level

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