Levels of Serum Phosphorus and Cardiovascular Surrogate Markers
A Population-Based Cross-Sectional Study

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Aim: This study aimed to evaluate the cross-sectional association between serum phosphorus and arterial stiffness among a health checkup population.

Methods: The study population included 26791 individuals without impaired kidney function. Arterial stiffness was measured by brachial-ankle pulse wave velocity (baPWV), ankle brachial index (ABI), and augmentation index (AI) by the radial artery waveform analysis. Linear or logistic regression model was used to appropriately evaluate the association between phosphorus levels and arterial stiffness markers.

Results: The mean age of the population was 49 years and 67% were male. The phosphorus level was divided into quintiles. After multivariate adjustments, participants in the fourth (3.90-4.17 mg/dL) and fifth quintile (≥4.18 mg/dL) of serum phosphorus had increased the level of baPWV with linear regression coefficients of 11.9 [95% confidence interval (CI): 5.6-18.2] and 17.2 (95% CI: 10.9-23.5), respectively, compared with those in the first quintile (<3.34 mg/dL). No significant associations were found between each quintile of phosphorus and ABI. However, participants in the fifth quintile of phosphorus had an increased risk of ABI ≥1.3 with an odds ratio (OR) of 1.2 (95% CI: 1.0-1.5) compared with the reference quintile. Furthermore, the increased risks could be observed for AI >97% throughout the second to fifth quintile of phosphorus and the ORs were 1.1 (95% CI: 1.0-1.3), 1.2 (95% CI: 1.0-1.4), 1.3 (95% CI: 1.1-1.5), and 1.5 (95% CI: 1.3-1.7), respectively.

Conclusions: Higher serum phosphorus levels, even within the normal range, are associated with markers of arterial stiffness among general population with normal kidney function.

J Atheroscler Thromb, 2016; 23: 95-104.

Key words: Ankle-brachial index, Arterial stiffness, Augmentation index, Brachial-ankle pulse wave velocity, Serum phosphorus

Introduction

Cardiovascular disease (CVD) is the leading cause of death and disabilities worldwide¹, ². Besides established risk factors for CVD, including hypertension and diabetes, recent studies indicated that the levels of serum phosphorus were independently associated with the risk of CVD in patients with chronic kidney disease (CKD)³, as well as in the general population⁴-⁶.

Experimental studies indicated that the phosphate load could induce vascular smooth muscle cells to change into a chondrocyte or osteoblast-like cell, and therefore, induced mineral deposition within the medial layer of the large and medium sized arteries⁷. Vascular calcification results in increased arterial stiffness⁸, which could be detected by several cardiovascular surrogate markers including ankle brachial index
(ABI), brachial-ankle pulse wave velocity (baPWV), and augmentation index (AI)\(^9\).

**Aim**

Previous studies investigating the association between levels of serum phosphorus and markers of arterial stiffness in general populations were limited and yielded inconsistent results\(^{10-14}\). Therefore, we initiated the present cross-sectional study to evaluate the association of serum phosphorus with ABI, baPWV, and AI in the general Chinese population with normal kidney function.

**Methods**

**Study Population**

From October 2010 to October 2012, there were 31,079 participants aged \( \geq \) 18 years attending the health examination with a total of 35,114 records at the Health Management Institute of the Chinese People’s Liberation Army General Hospital. The participants were mainly institutionalized employees from government and public institutions in Beijing, as well as some individual customers. The data of the first examination were used if repeated health examinations were made in the study period. Some participants did not receive vascular stiffness testing (ABI, baPWV, or AI) \((n=3357)\) and some participants were with incomplete data on basic examinations (height, weight, systolic blood pressure, diastolic blood pressure, hemoglobin, fasting glucose, blood lipids, serum phosphorus, or serum creatinine) \((n=1009)\). We also excluded participants with impaired estimated glomerular filtration rate (eGFR) \(< 60 \text{ ml/min/1.73 m}^2\) \((n=122)\), which was estimated by serum creatinine with an adapted equation of the Modification of Diet in Renal Disease equation on the basis of data from Chinese CKD patients\(^15\). Altogether, 26,791 participants were included in the final analysis. Individuals excluded from the study were tended to be older (mean age, 51.0 vs 49.0 years) and consisted of less male patients (male percentage, 60.2% vs 66.9%) than those included in the study. Each participant signed an informed consent authorizing the use of data for scientific research. The study protocol was approved by the Ethics Committee of the Peking University First Hospital. Data were kept anonymous during the entire study process.

**Anthropometric and Laboratory Measurements**

General information was inquired through questionnaires. Height and weight were measured with light clothes and without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Blood pressure was measured by a trained physician with the mercury sphygmomanometer (Yuyue, Armamentarium Limited Company, Jiangsu, China). Participants were required to rest in a supine position for at least 5 min before measurement. The blood pressure was measured three times at \( \geq \) 1-min intervals. The mean value of the blood pressure was calculated. The mean arterial pressure (MAP) was calculated as one third of the systolic blood pressure plus two thirds of the diastolic blood pressure.

Venous blood specimens were collected after an overnight fast. Concentrations of serum phosphorus, fasting glucose, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using a Roche COBAS Integra 800 Automated Analyzer (Roche Diagnostics, Indianapolis, IN, USA) and serum hemoglobin (HGB) was measured using a Coulter LH 750 Hematology Analyzer (Beckman Coulter, Brea, CA, USA) in the Department of Biochemistry, PLA General Hospital. The reportable range of serum phosphorus was 0.5-20 mg/dL, and inter- and intra-assay coefficients of variation were \( \leq 3\%\). Albuminuria was measured using immunoturbidimetric tests and urinary creatinine with Jaffe’s kinetic method by using a fresh morning spot urine sample. The urinary albumin to creatinine ratio (ACR; mg/g creatinine) was calculated and divided into A1 \((< 30 \text{ mg/g})\), A2 \((30-299 \text{ mg/g})\), and A3 \((\geq 300 \text{ mg/g})\). Serum creatinine was measured using a Hitachi 7600 Auto-analyzer (Hitachi, Tokyo, Japan). The albuminuria, urinary creatinine, and serum creatinine levels were measured in the laboratory of Nephrology Department, PLA General Hospital.

**Measurements of ABI, baPWV, and AI**

The ABI and baPWV were measured non-invasively by using the BP-203RPEIII automatic waveform analyzer (Omron Healthcare Co., Ltd., Kyoto, Japan). The detailed procedure was introduced previously\(^16\). In brief, the pulse waves of the right brachial artery and both tibial arteries were detected by pressure transducers and recorded by the oscillometric method. Therefore, two baPWV and ABI values in each person were obtained, and we used the mean value of baPWV and lower value of ABI as the index value for analysis. The oscillometric method showed good correlations as compared with the traditional manual methods, such as the auscultation and Dop-
plier method\textsuperscript{17}. AI was measured by the HEM-9000AI automated tonometer device (Omron Healthcare Co., Ltd., Kyoto, Japan). The validity of the method was as good as the catheter method\textsuperscript{18}. All measurements were performed in the morning by trained physicians. The intra-class correlations between physicians performing the measurements were shown to be 0.80 in a test for 10 participants.

Statistical Analysis

Data are presented as proportions for categorical variables and as mean ± SD (standard deviation) for continuous variables except for highly skewed variables, which is presented as median (inter-quartile range, IQR). The levels of serum phosphorus were divided into quintiles with the levels of <3.34 mg/dL, 3.34-3.64 mg/dL, 3.65-3.89 mg/dL, 3.90-4.17 mg/dL, and ≥4.18 mg/dL, respectively. Relevant characteristics were described and compared according to the quintiles of serum phosphorus. One-way ANOVA or Kruskal-Wallis test was used for comparison of continuous variables and the Chi-square test for binary variables.

High baPWV was defined as values of >75\% percentile (1466 cm/s) in the study population. The multivariable linear regression model was used to analyze the association between the levels of serum phosphorus and baPWV as a continuous variable. ABI was analyzed as categorical variables (<0.9, 0.9-1.3, and ≥1.3)\textsuperscript{19}, and multinomial logistic regression model was used to analyze the association between the levels of serum phosphorus and ABI. For AI, the values of >90\% percentile (97\%) in the study population were considered as abnormal, and logistic regression model was used in the analyses. The quintiles of phosphorus were treated as four dummy variables in the regression models and the bottom quintile of phosphorus was used as a reference. Variables included in regression analyses were demographics (age and sex), traditional cardiovascular risk factors (BMI, heart rate, MAP, HGB, LDL-C, HDL-C, and fasting glucose), and markers of kidney damage (eGFR and ACR). Besides sex (male vs female) and ACR (A1, A2, and A3), all covariates were treated as continuous variables with the skewed variables (fasting glucose and eGFR) in logarithm. The regression coefficient (\(\beta\)) with standard error (SE) or odds ratio (OR) with 95\% confidence interval (CI) were reported. All analyses were conducted by SAS software (version 9.1, SAS institute, CA, USA). The \(P\) value of 0.05 was considered as statistically significant.

Results

Altogether, 26,791 participants with an average age of 49.0 ± 8.9 years and male predominance (66.9\%) were included. The mean (full range) serum phosphorus level was 3.8±0.5 mg/dL (2.1-5.0 mg/dL), and the mean baPWV, ABI, and AI were 1350.8±224.0 cm/s (767.0-2839.5 cm/s), 1.11 ± 0.08 (0.59-1.59), and 81.2±13.2\% (32-139\%), respectively. The characteristics of the study population stratified by quintiles of serum phosphorus levels are shown in Table 1. Compared with those with lower levels of phosphorus, participants in the higher quintiles were younger; had a lower percentage of males; high baPWV and ABI; higher percentage of AI abnormality; lower percentage of individuals in the A2 and A3 stages of ACR; lower levels of MAP, fasting glucose, HGB, baPWV, and ABI; and higher levels of TC, LDL-C, HDL-C, eGFR, and AI.

People with ABI <0.9 were excluded from the analysis for baPWV, because baPWV would no longer reflect arterial stiffness in the participants with severe arterial stenosis. The multivariable-adjusted regression coefficient for baPWV was \(-5.0\) (95\% CI: \(-11.1-1.1\)) in the second quintile than in the first quintile. The value increased to 11.9 (95\% CI: 5.6-18.2) and 17.2 (95\% CI: 10.9-23.5) when the fourth and fifth quintiles were compared with the first quintile, respectively. The association of serum phosphorus and baPWV was in an opposite direction compared with the results listed in Table 1. Age and mean arterial pressure were mainly responsible for the negative confounding observed here. Data are shown in Table 2.

For ABI categories, the significant association was observed in the fully adjusted model of the fifth quintile of serum phosphorus with a high ABI (≥1.3) compared with the first quintile. The OR value was 1.2 (95\% CI: 1.0-1.5). From the second to the fourth quintile of serum phosphorus, the OR values increased from 0.8 (95\% CI: 0.6-1.0) and 1.0 (95\% CI: 0.8-1.3) to 1.2 (95\% CI: 0.9-1.4), but were not statistically significant. No significant associations were found between each quintile of phosphorus and low ABI (<0.9) (Table 3).

In the fully adjusted model, the levels of serum phosphorus were independently associated with AI abnormality, starting from the second quintile to the fifth quintile of serum phosphorus with ORs of 1.1 (95\% CI: 1.0-1.3), 1.2 (95\% CI: 1.0-1.4), 1.3 (95\% CI: 1.1-1.5), and 1.5 (95\% CI: 1.3-1.7), respectively (\(P<0.01\)) (Table 4).

Only 56.5\% of our participants (\(n=15141\)) had information of past CVD. To investigate the potential
Table 1. Characteristics of participants according to serum phosphorus levels

| Characteristic                        | Serum phosphorus (mg/dL) | p value |
|---------------------------------------|--------------------------|---------|
|                                       | <3.34 (n= 4993)          |         |
|                                       | 3.34-3.64 (n=5092)       |         |
|                                       | 3.65-3.89 (n=5383)       |         |
|                                       | 3.90-4.17 (n=5124)       |         |
|                                       | >4.18 (n=5443)           |         |
| Age, years                            | 50.3±9.0                 |         |
| Male, no.                             | 3755 (76.0%)             |         |
| BMI, kg/m²                            | 25.4±3.1                 |         |
| Heart rate, beats/min                 | 72.1±10.0                |         |
| MAP, mmHg                             | 94.5±12.8                |         |
| Fasting glucose, mmol/L              | 5.4 (5.0, 6.0)           |         |
| TC, mmol/L                            | 4.9±1.0                  |         |
| LDL-C, mmol/L                         | 3.0±0.8                  |         |
| HDL-C, mmol/L                         | 1.2±0.3                  |         |
| TG, mmol/L                            | 1.5 (1.0, 2.2)           |         |
| HGB, g/L                              | 150.0±15.7               |         |
| eGFR, ml/min/1.73 m²                 | 111.6 (98.4, 126.9)      |         |
| baPWV, cm/s                           | 1377.8±239.5             |         |
| ABI                                   | 1.114±0.079              |         |
| Al %                                  | 79.8±12.9                |         |
| High baPWV⁴                           | 1455 (29.5%)             |         |
| High ABI⁵                             | 64 (1.3%)                |         |
| Al abnormality⁶                        | 413 (8.4%)               |         |
| ACR, mg/g                             | 4102 (83.1%)             |         |
| <30                                   | 5006 (84.8%)             |         |
| 30-299                                | 217 (4.4%)               |         |
| >300                                  | 36 (0.7%)                |         |
| missing                               | 584 (11.8%)              |         |

Values are presented as number (%), mean value ± SD, or median (interquartile range). *High baPWV was defined as the value of >75% percentile in the study population. †High ABI was defined as the value of >1.3. ‡Al abnormality was defined as the value of >90% percentile in the study population. BMI = body mass index; MAP = mean arterial pressure; TC = total cholesterol; LDL-C = low density lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol; TG = triglyceride; HGB = hemoglobin; GFR = estimated glomerular filtration rate; baPWV = brachial-ankle pulse wave velocity; ABI = ankle brachial index; Al = augmentation index; ACR = albumin creatinine ratio.

Table 2. Regression coefficient for the association between serum phosphorus and baPWV*

| Serum phosphorus quintiles(mg/dL) | Age and sex adjusted change of baPWV (95%CI) | Fully adjusted change of baPWV (95%CI) |
|-----------------------------------|---------------------------------------------|----------------------------------------|
| <3.34                             | reference                                    | reference                              |
| 3.34-3.64                         | -11.8 (-19.1, -4.5)                         | -5.0 (-11.1, 1.1)                      |
| 3.65-3.89                         | -4.6 (-12.0, 2.8)                           | 3.2 (-3.1, 9.5)                        |
| 3.90-4.17                         | 0.5 (-6.9, 7.9)                             | 11.9 (5.6, 18.2)                       |
| ≥ 4.18                            | 4.2 (-3.2, 11.6)                            | 17.2 (10.9, 23.5)                      |

*Participants with ABI <0.9 were excluded with the final sample size of 26747. †Adjusted for age, sex, body mass index, heart rate, mean arterial pressure, hemoglobin, low density lipoprotein cholesterol, high density lipoprotein cholesterol, logarithm of fasting glucose, logarithm of estimated glomerular filtration rate, and categories of albumin creatinine ratio. Except for hemoglobin and low density lipoprotein cholesterol, the regression coefficients of all other covariates in the fully adjusted model were statistically significant. baPWV = brachial-ankle pulse wave velocity; CI = confidence interval.

In our study, we found that the serum phosphorus levels were associated with multiple markers of arterial stiffness among the general population with a normal kidney function. A dose-response relationship could be observed for phosphorus levels and Al starting from the second quintile of phosphorus levels. The association between higher levels of phosphorus and effect of people with CVD history on the association between serum phosphorus and arterial stiffness markers, we performed a sensitivity analysis among the participants who had reported not having a CVD history (95.0%, n=14378). In the fully adjusted model, the associations between serum phosphorus and the three markers were all not materially changed. We listed the result in the supplemental table.

Discussion

In our study, we found that the serum phosphorus levels were associated with multiple markers of arterial stiffness among the general population with a normal kidney function. A dose-response relationship could be observed for phosphorus levels and Al starting from the second quintile of phosphorus levels. The association between higher levels of phosphorus and
The underlying pathogenesis of arterial calcification be the de-differentiation of vascular smooth muscle cells to osteoblast/chondrocyte like cells\(^20\). The calcification that occurred in the medial layer can decrease the compliance of the vessel, while the calcification of the intimal layer can be commonly detected in the late stage of atherosclerosis. Recent studies have even reported that arterial calcification could be found in the earlier course of atherosclerosis as a circumferential lesion\(^21\). Actually, arterial stiffness may share a lot of cardiovascular risk factors with atherosclerosis, such as age, sex, hemoglobin A1c, systolic blood pressure, and serum cholesterol, as shown in a study in patients with type 2 diabetes mellitus exploring the risk factors for baPWV and intima-media thickness\(^22\). Furthermore, the locations of stiffening have some differences between the selected markers. baPWV reflected arterial stiffness in both peripheral and central arteries. ABI provides an estimation of obstruction in peripheral arteries with its value \(\leq 0.9\) as a standard cutoff value for peripheral artery disease. Meanwhile, the increased value of ABI was noted as a signal reflecting a relatively incompressible low extreme arterial wall\(^23\). Studies about AI were scarce. It was a measure of pulse wave reflection back to the heart and can be used as a marker reflecting the stiffness of large and central arteries\(^9\).

| Table 3. Odds ratio for the association between serum phosphorus and ABI categories |
|-----------------------------------|----------------|----------------|----------------|
| Serum phosphorus quintiles (mg/dL)| ABI<0.9 (n=44) | ABI: 0.9-1.3 (n=26405) | ABI>1.3 (n=342) |
| Age and sex adjusted OR (95% CI) |                |                |                |
| <3.34 (reference)                | 1.0            | 1              | 1.0            |
| 3.34-3.64                        | 0.7 (0.3, 1.4) | 1              | 0.8 (0.6, 1.0) |
| 3.65-3.89                        | 1.3 (0.7, 2.3) | 1              | 1.0 (0.8, 1.2) |
| 3.90-4.17                        | 0.9 (0.5, 1.7) | 1              | 1.2 (1.0, 1.5) |
| \(\geq 4.18\)                    | 0.9 (0.5, 1.6) | 1              | 1.2 (1.0, 1.5) |
| \(p\) for trend                  | 0.36           | -              | 0.27           |
| Fully adjusted OR (95% CI)*      |                |                |                |
| <3.34 (reference)                | 1.0            | 1              | 1.0            |
| 3.34-3.64                        | 0.7 (0.3, 1.4) | 1              | 0.8 (0.6, 1.0) |
| 3.65-3.89                        | 1.3 (0.8, 2.3) | 1              | 1.0 (0.8, 1.3) |
| 3.90-4.17                        | 0.9 (0.5, 1.7) | 1              | 1.2 (0.9, 1.4) |
| \(\geq 4.18\)                    | 0.9 (0.5, 1.6) | 1              | 1.2 (1.0, 1.5) |
| \(p\) for trend                  | 0.45           | -              | 0.38           |

\(^{*}\)Adjusted for age, sex, body mass index, heart rate, mean arterial pressure, hemoglobin, low density lipoprotein cholesterol, high density lipoprotein cholesterol, logarithm of fasting glucose, logarithm of estimated glomerular filtration rate, and categories of albumin creatinine ratio. The odds ratios of age, sex, body mass index, systolic blood pressure, diastolic blood pressure, high density lipoprotein cholesterol, and logarithm of fasting glucose were statistically significant for ABI \(\geq 1.3\), while only age and sex were statistically significant for ABI <0.9. ABI= ankle brachial index; OR= odds ratio; CI= confidence interval.

| Table 4. Association between serum phosphorus and AI abnormality |
|-----------------------------------|----------------|----------------|
| Serum phosphorus quintiles (mg/dL)| Age and sex adjusted OR (95% CI) | Fully adjusted OR (95% CI)* |
|                                   |                |                |
| <3.34 (reference)                | 1.0            | 1.0            |
| 3.34-3.64                        | 1.1 (1.0,1.3)  | 1.1 (1.0,1.3)  |
| 3.65-3.89                        | 1.2 (1.1,1.4)  | 1.2 (1.0,1.4)  |
| 3.90-4.17                        | 1.3 (1.1,1.5)  | 1.3 (1.1,1.5)  |
| \(\geq 4.18\)                    | 1.5 (1.3,1.7)  | 1.5 (1.3,1.7)  |
| \(p\) for trend                  | \(<0.01\)      | \(<0.01\)      |

\(^{*}\)Adjusted for age, sex, body mass index, heart rate, mean arterial pressure, hemoglobin, low density lipoprotein cholesterol, high density lipoprotein cholesterol, logarithm of fasting glucose, logarithm of estimated glomerular filtration rate, and categories of albumin creatinine ratio. Except for the low density lipoprotein cholesterol, logarithm of estimated glomerular filtration rate, and the categories of albumin creatinine ratio, the odds ratios of all other covariates were statistically significant. AI= augmentation index; OR= odds ratio; CI= confidence interval.

baPWV and ABI \(\geq 1.3\) were also observed, but no association between phosphorus and ABI <0.9 was found.

baPWV, ABI, and AI are all surrogate markers for arterial stiffness and arterial calcification, which could happen in all arterial beds and both the intimal and medial layer of vessel wall. Studies suggested the
PWV was considered as an important surrogate marker for arterial stiffness. However, studies exploring the relationship between serum phosphorus and PWV produced inconsistent results. One study including 57 CKD patients under the peritoneal dialysis treatment did not find the correlation of serum phosphorus, as well as calcium and intact parathyroid hormone, with aorticPWV. However, the other study, which also focused on peritoneal dialysis patients, observed a significant correlation (correlation coefficient=0.324, \( P=0.008 \)) between serum phosphorus and hPWV (heart to femoral PWV) during 1 year of follow-up. In a study conducted in the community-dwelling elderly, Madero et al claimed that an inverse association of each 0.48 mg/dL is higher in serum phosphorus with 1.77 cm/s lower aortic PWV. The inconsistency of the previous studies may be attributed to the different characteristics of the study population (age distribution, serum phosphorus levels, and with or without CKD), study design (cross-sectional or cohort study), and insufficient sample size. baPWV is the nearest value to the traditional aortic PWV. Our study used a large sample of health examination population with a wide age range and healthier than those used in previous studies after removing patients with impaired kidney function and those who showed a positive association between serum phosphorus in a high normal level (>3.90 mg/dL) and baPWV. The null association in the age and sex-adjusted model may be due to the confounding effect of MAP and other non-included confounding factors. However, the corresponding change of baPWV with the increase of serum phosphorus was still very small (10.7 cm/s or 16.6 cm/s) and may lack clinical significance.

Many studies exploring the association of serum phosphorus and peripheral arterial stiffness used ABI as the marker. Kendrick et al demonstrated a strong, positive association (OR=4.78, 95% CI: 1.73-13.2) between serum phosphorus (3.7-5.0 mg/dL) and ABI >1.3 compared with the reference phosphorus level of 3.1-3.4 mg/dL using the data from the Third National Health and Nutrition Examination Survey (NHANES) of USA. In the Multi-Ethnic Study of Atherosclerosis (MESA), where participants were older (aged 45-84) and a quarter of them were with moderate kidney disease, participants with a phosphorus level of >4 mg/dL had an increased risk of suffering a high ABI (>1.3) (OR=4.6, 95% CI: 1.6-13.2) compared with those with a phosphorus level of <3 mg/dL. However, another study conducted in older men found that the existence of an association between phosphorus levels and high ABI depended on the CKD affected status. In persons without CKD, the positive association disappeared. In addition to the previous studies, we observed that the serum phosphorus levels within the normal range could still increase the risk of arterial stiffness (ABI ≥ 1.3), but the magnitude of the effect was small. However, we did not observe a significant association between increased phosphorus levels and ABI <0.9. The small value of effect for ABI ≥ 1.3 and the null association for ABI <0.9 may be due to the limited sample size of people with an ABI <0.9 in our study.

In a previous cross-sectional study with 74 patients with stage 3-4 CKD, no associations of serum phosphorus, as well as dietary phosphorus intake, urinary phosphate excretion, and fibroblast growth factor 23, were found with AI. However, this study has a relatively small sample size, which may limit the power to detect small to modest effects. To the best of our knowledge, no study has been conducted evaluating the effect of increased serum phosphorus and AI in the general population. In our study with a large sample of general population, we found significant positive associations of the phosphorus level with high AI and the increased risk started from the second quintile of serum phosphorus, which was different from that in the fourth or fifth quintile of serum phosphorus for baPWV and ABI. Many potential confounding factors influencing AI were adjusted, including the heart rate, which was tested to reduce AI by 4.8% for each 10 beats per min increment. In the analysis, we observed inconsistent associations for baPWV and AI in the quintile 1-3 of serum phosphorus, although the two markers should have a positive correlation. We assumed the reason for the discrepancy was due to the different confounders for each index.

Our study had some advantages, such as the large sample size and inclusion of several arterial stiffness markers simultaneously. However, some limitations needed to be acknowledged. This study was based on a cross-sectional data; hence, the causal effect of serum phosphorus on arterial stiffness cannot be demonstrated. Furthermore, several biomarkers for mineral metabolic disorder and arterial inflammation were not included, such as serum calcium, intact parathyroid hormone, 1,25-dihydroxyvitamin D, and fibroblast growth factor-23; therefore, we cannot evaluate their effect on vascular stiffness as confounding factors. Finally, only 56.5% of our participants had information of past CVD. However, given the results of sensitivity analysis among the participants without CVD, it is unlikely that the presence of CVD would have major impacts on our results.
Conclusion

Our study found higher serum phosphate concentrations, which were within the normal range, associated with an increased value of baPWV and ABI ≥ 1.3 and showed a dose-response effect of AI, but no associations were found for ABI < 0.9 in the general population. Further longitudinal studies with a large sample size and comprehensive measurement of these markers were warranted to evaluate the association between abnormal mineral metabolism and arterial stiffness.

Acknowledgements

The research support for this study was provided by National Key Technology R&D Program of the Ministry of Science and Technology (2011BAI10B01); Establishment of early Diagnosis pathway and model for evaluating progression of chronic kidney disease (D131100004713007) from the Beijing Science and Technology Committee.

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The authors declare no conflict of interest.

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Supplemental Table 1. Regression coefficient, odds ratio and 95%CI for the association between serum phosphorus and the arterial stiffness markers in the fully adjusted model among the population with normal kidney function and without a history of cardiovascular diseases (n = 14 378)*

| Serum phosphorus quintiles(mg/dL) | Change of baPWV | ABI<0.9 versus ABI in 0.9-1.3 | ABI ≥ 1.3 versus ABI in 0.9-1.3 | AI abnormality |
|-----------------------------------|-----------------|-------------------------------|---------------------------------|----------------|
| <3.34                             | reference       | 1 (reference)                 | 1 (reference)                   | 1 (reference) |
| 3.34-3.64                         | -5.6 (-13.2, 2.0) | 0.3 (0.1, 2.1)               | 0.7 (0.5, 1.0)                  | 1.0 (0.9, 1.3) |
| 3.65-3.89                         | 1.4 (-6.4, 9.2)  | 1.9 (0.7, 5.3)               | 1.0 (0.7, 1.4)                  | 1.1 (0.9, 1.4) |
| 3.90-4.17                         | 12.2 (4.2, 20.2) | 0.4 (0.1, 2.3)               | 1.4 (1.1, 1.9)                  | 1.2 (1.0, 1.5) |
| ≥ 4.18                            | 16.8 (8.8, 24.8) | 1.9 (0.7, 5.3)               | 1.4 (1.1, 1.9)                  | 1.6 (1.3, 1.9) |

Data are presented as regression coefficient (95%CI) or odds ratio (95%CI). *Adjusted for age, sex, body mass index, heart rate, mean arterial pressure, hemoglobin, low density lipoprotein cholesterol, high density lipoprotein cholesterol, logarithm of fasting glucose, logarithm of estimated glomerular filtration rate, and categories of albumin creatinine ratio. baPWV=branchial-ankle pulse wave velocity; ABI=ankle brachial index; AI=augmentation index; CI=confidence interval.