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ORIGINAL ARTICLE
Metabolic Disorder

Deterioration of Phosphate Homeostasis Is a Trigger for Cardiac Afterload

— Clinical Importance of Fibroblast Growth Factor 23 for Accelerated Aging —

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Background: After the discovery of the Klotho gene, phosphate came into focus as a pathogenetic aging agent. Phosphate homeostasis is controlled by phosphate-regulating hormones: fibroblast growth factor 23 (FGF23), vitamin D_3, and parathyroid hormone. This study investigated the relationship between the deterioration in phosphate homeostasis and arterial stiffness by measuring serum FGF23 concentrations.

Methods and Results: The study subjects comprised 82 hospitalized patients (31 males, 51 females; mean [±SD] age 78.6±10.5 years). All patients underwent chest computed tomography, measurement of central blood pressure (BP), and blood chemistry tests. Arterial calcification and/or stiffness was evaluated using the Agatston calcification score (ACS) and pulse wave velocity (PWV). PWV was significantly correlated with age (t=23.47, P<0.0001), estimated glomerular filtration rate (eGFR; t=−4.40, P<0.0001), and ACS (t=4.36, P<0.0001). Serum FGF23 concentrations were significantly correlated with age (t=2.52, P=0.014), eGFR (t=−3.37, P<0.001), serum inorganic phosphorus concentrations (t=3.49, P<0.001), serum vitamin D_3 concentrations (t=−4.57, P<0.001), ACS (t=2.30, P=0.025), augmentation pressure (t=2.48, P=0.015), central systolic BP (t=2.00, P=0.049), plasma B-type natriuretic peptide (BNP) concentrations (t=3.48, P<0.001), and PWV (t=2.99, P=0.004). PWV was positively related to augmentation pressure (t=4.09, P<0.001), central systolic BP (t=3.13, P=0.002), and plasma BNP concentrations (t=3.54, P<0.001).

Conclusions: This study shows that the increase in serum FGF23 concentrations reflects deterioration of phosphate homeostasis and is an important predictor for arterial stiffness, which intensifies cardiac afterload.

Key Words: Arterial stiffness; Afterload; Calcification; Fibroblast growth factor 23; Phosphate

Aging is a multifactorial process often characterized by a progressive decline in physiological functions. Accelerated aging is accompanied by a high prevalence of cardiovascular risk factors, such as arterial calcification, atherosclerosis, chronic kidney disease (CKD), hypertension, left ventricular hypertrophy, heart failure, and osteoporosis, all of which increase cardiovascular morbidity and mortality.[1,2]

The Klotho/fibroblast growth factor 23 (FGF23) axis is an important regulating factor of aging, including cardiovascular mortality.[1–4] Accumulation of phosphate has recently been focused on as a pathological aging factor.[1–4] The serum phosphate concentration is controlled within a small range, with phosphate homeostasis maintained by a counterbalance between the absorption of dietary phosphate from the intestines and the excretion of blood phosphate from the kidneys into the urine.[1–5] FGF23 is the most potent hormone regulating phosphate homeostasis.[4–9] It promotes phosphate excretion and acts on the kidneys, where Klotho is mostly expressed.[1,2,4–9] Vitamin D_3 is physiologically important and is activated in the kidney; it acts on the intestine to increase the

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absorption of phosphate and calcium, thereby inducing a positive phosphate balance.[6–9] Accumulation of phosphate and phosphate-related hormones could induce pathological arterial calcification.[1,2,4–9] Vitamin D_3 is a physiologically important hormone active against osteoporosis, atherosclerosis, endothelial dysfunction, inflammation, and left ventricular hypertrophy.[10,11] Parathyroid hormone (PTH) is secreted from the parathyroid gland in response to decreases in serum calcium and phosphate concentrations, and promotes phosphate excretion into the urine under the control of FGF23.[4,8,9]

It is not known whether accumulation of phosphate and phosphate-related hormones may induce pathological arterial stiffness. In this study we investigated whether deterioration in phosphate homeostasis induced arterial stiffness and increased cardiac afterload by using computed tomography (CT), central blood pressure (BP) measurements, and blood sampling tests.

Methods

Study Subjects

This prospective cross-sectional study comprised 82 consecutive patients (31 males, 51 females; mean [±SD] age 78.6±10.5 years) who were admitted or referred to our institution or outpatient clinic with suspected cardiovascular diseases between April 1, 2018 and March 31, 2022 (**Tables 1**,**2**). None of the patients was on hemodialysis. All females in the study were postmenopausal and none was on hormone replacement therapy.

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of our institution (Approval no. JMC 269-2108).

Assessments of Pulse Wave Velocity (PWV) and Central BP Measurements

We used PWV as a measure of arterial stiffness. Arterial stiffness was detected using the Mobil-O-Graph pulse wave analysis (PWA)/ambulatory BP monitoring device (I.E.M. GmBH, Stolberg, Germany), which is a cuff-based oscillometric method of measurement. The device has been approved for BP measurement by the British Hypertension Society and European Society of Hypertension, and device reliability has been demonstrated in comparison with invasive and non-invasive methods for PWA.[12,13] BP measurements were performed on patients' left upper arms after a 10-min rest. Patients were seated and had their elbow flexed and supported at heart level on the chair. Augmentation pressure, augmentation index, and central BP, including general brachial artery BP measurements, were also measured. Augmentation pressure is the additional aortic systolic pressure produced by the return of the reflected waves at the central aorta.

Thoracic Aorta Calcification (TAC) Score

The burden of TAC was determined from each participant's CT scan, from the aortic annulus, above the aortic valve, to the lower edge of the pulmonary artery bifurcation (ascending aorta), as well as from the lower edge of the pulmonary bifurcation to the cardiac apex (descending aorta). A TAC score was determined for each study participant using the Agatston score and calcification volume score, a method that has been widely used as a convenient method to quantify TAC.[14] The aortic arch could not be visualized in the available CT sections; therefore, calcifications in the arch were not taken into account when calculating the TAC score.

Blood Chemistry Measurements

Blood samples for measurement of clinical chemistry and other data were collected from patients in a supine position after an overnight fast. Biochemical and other analyses were performed using standard laboratory procedures. Venous blood samples were obtained at enrollment, processed, and then stored at −80°C until assay.

Serum FGF23 concentrations were measured by an ELISA that recognizes only full-length biologically active FGF23 and has a detection limit of 3 pg/mL (Kainos, Tokyo, Japan). The reference range of FGF23 in healthy adults measured by this ELISA is 10–50 pg/mL with a mean value of about 30 pg/mL.[15]

Levels of an active form of vitamin D, namely 1,25-dihydroxyvitamin D (vitamin D_3), were determined at baseline with a fully automated and sensitive immunoassay that uses a recombinant fusion construct of the vitamin D receptor ligand-binding domain for the specific capture of vitamin D_3 (DiaSorin, Saluggia, Italy). The limit of quantification for this vitamin D_3 assay was 5 pg/mL and the reference interval determined in healthy volunteers ranges between 25.0 and 86.5 pg/mL, with a median of 48.1 pg/mL.[16]

The intact PTH assay was performed using Allegro Intact PTH (I-Nichols, San Juan Capistrano, CA, USA). Normal values range from 10 to 65 pg/mL.[17]

Plasma B-type natriuretic peptide (BNP) concentrations were measured using a specific immunoradiometric assay for human BNP (TOSOH Corp., Tokyo, Japan). The minimum detectable quantity of human BNP was 2.0 pg/mL. The mean intra- and interassay coefficients of variation were 2.3% and 3.0%, respectively.

Statistical Analysis

Baseline clinical data are expressed as the mean±SD or median with interquartile range (IQR) for continuous variables, and the significance of differences within the group were evaluated using either unpaired t-tests or the Mann-Whitney rank-sum test. For discrete variables, data are expressed as counts and percentages and were analyzed using the Chi-squared test. The classification of "habitual smoking" included current and past smokers. History of atrial flutter (AF) included past transient AF experiences. Linear regression analysis was used to assess the association between each parameter. The linearity of relationships between the response variable and different predictor variables was evaluated using linear regression analysis with t-tests. For logistic regression analysis, PWV and phosphate concentrations were each divided into 2 groups ("lower" and "higher") based on their medium value.

In all cases, 2-tailed $P<0.05$ was considered statistically significant. Statistical analyses were performed using STATA 17.0 (STATA Corp., College Station, TX, USA).

Results

Clinical Characteristics in the Lower and Higher PWV Groups

Study subjects were divided into 2 groups according to the medium PWV values (i.e., 11.95 m/s), as shown in **Table 1**. Compared with the lower PWV group, the higher PWV group had significantly higher age, creatinine, blood urea nitrogen, serum FGF23 and high-sensitivity C-reactive

Table 1. Clinical Parameters in the Higher and Lower PWV Groups

	PWV group		P value
	Lower (n=41)	Higher (n=41)	
Age (years)	71.6±10.1	85.5±4.7	<0.001
Male sex	18 (43.9)	13 (31.7)	0.25
BMI (kg/m²)	23.5±3.6	22.5±4.5	0.30
SBP (mmHg)	119.9±14.8	131.2±19.8	0.005
DBP (mmHg)	68.7±9.4	68.3±14.0	0.87
Pulse pressure (mmHg)	51.2±10.9	62.9±17.3	0.0004
Heart rate (beats/min)	67.5±11.0	70.6±16.0	0.32
Blood tests			
Albumin (g/dL)	3.85±0.43	3.47±0.54	<0.001
Glucose (mmol/L)	99 [92–115]	99 [87–113]	0.43
HbA1c (%)	6.0 [5.6–6.5]	6.0 [5.6–6.4]	0.84
Creatinine (mg/dL)	0.86±0.23	1.10±0.74	0.048
BUN (mg/dL)	17.5±6.4	24.2±11.2	0.002
TC (mmol/L)	187 [163–209]	171 [156–202]	0.21
LDL-C (mmol/L)	100 [88–124]	86 [63–115]	0.09
HDL-C (mmol/L)	59 [42–69]	63 [48–78]	0.32
Triglycerides (mmol/L)	102 [78–136]	100 [75–122]	0.51
AST (U/L)	21 [18–27]	22 [18–27]	0.66
ALT (U/L)	14 [10–19]	12 [8–16]	0.11
γGTP (U/L)	23 [17–41]	20 [15–40]	0.23
Leukocyte (/μL)	5,500 [4,500–6,000]	5,100 [3,800–6,600]	0.85
Hemoglobin (g/dL)	13.0 [12.4–14.7]	11.1 [9.8–12.2]	<0.001
eGFR (mL/min/1.73 m²)	61.2±16.2	50.1±20.0	0.017
PTH (intact) (pg/mL)	40 [32–61]	48 [32–64]	0.53
1,25(OH)₂VD (pg/mL)	47.0 [35.0–57]	33.0 [23.0–46.0]	0.008
FGF23 (pg/mL)	39.5 [32.9–46.2]	48.9 [38.7–79.1]	0.014
Calcium (mg/dL)	8.91±0.42	8.81±0.56	0.20
Inorganic phosphorus (mg/dL)	3.50±0.47	3.54±0.53	0.86
Magnesium (mg/dL)	2.27±0.14	2.19±0.27	0.30
hs-CRP (mg/dL)	0.06 [0.03–0.15]	0.28 [0.07–0.75]	<0.001
BNP (pg/mL)	45.9 [19.2–118.2]	129.8 [69.9–343.2]	<0.001
Thoracic CT			
Thoracic Agatston score (HU)	658 [311–1,846]	5,162 [2,326–9,532]	<0.001
Calcification volume (HU)	985 [339–2,581]	6,504 [2,932–11,629]	<0.001
Central BP index			
Reflection magnitude (%)	66 [61–74]	65 [59–70]	0.34
Augmentation index (%)	16 [11–30]	29 [20–38]	0.004
Augmentation pressure (mmHg)	6.0 [3.5–11.5]	12.0 [7.0–18.0]	0.004
PWV (m/s)	10.4 [9.4–11.2]	12.9 [12.3–13.8]	<0.001
Central SBP (mmHg)	108.8±16.0	116.0±18.3	0.06
Central DBP (mmHg)	73.9±12.5	74.0±14.3	0.97
Smoking habit			
Habitual smoker	16 (39.0)	11 (26.8)	0.24
Complications			
Hypertension	23 (56.1)	26 (63.4)	0.50
Diabetes	12 (29.3)	11 (26.8)	0.81
Hyperlipidemia	15 (35.6)	15 (35.6)	1.00

Unless indicated otherwise, data are given as the mean±SD, median [interquartile range], or n (%). 1,25(OH)₂VD, 1,25-dihydroxyvitamin D; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BNP, B-type natriuretic peptide; BP, blood pressure; BUN, blood urea nitrogen; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; γGTP, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; HU, Hounsfield units; LDL-C, low-density lipoprotein cholesterol; PWV, pulse wave velocity; SBP, systolic blood pressure; PTH, parathyroid hormone; SBP, systolic blood pressure; TC, total cholesterol.

Table 2. Clinical Parameters in the Low and High Serum FGF23 Groups

	FGF23 group		P value
	Low (n=41)	High (n=41)	
Age (years)	75.8±12.3	81.3±7.6	0.016
Male sex	16 (39.0)	15 (36.6)	0.82
BMI (kg/m²)	22.8±3.9	23.2±4.3	0.69
SBP (mmHg)	125.0±17.2	125.9±19.5	0.81
DBP (mmHg	67.2±10.4	70.9±16.3	0.30
Pulse pressure (mmHg)	55.1±11.9	58.8±18.3	0.29
Heart rate (beats/min)	67.2±10.4	70.9±16.3	0.24
Blood tests			
Albumin (g/dL)	3.78±0.48	3.55±0.54	0.044
Glucose (mmol/L)	99 [91–116]	99 [89–113]	0.66
HbA1c (%)	5.9 [5.6–6.7]	6.0 [5.5–6.5]	0.89
Creatinine (mg/dL)	0.79±0.18	1.18±0.72	0.0013
BUN (mg/dL)	17.0±4.9	24.7±11.7	0.0002
TC (mmol/L)	189 [168–215]	164 [146–195]	0.011
LDL-C (mmol/L)	111 [84–131]	93 [62–107]	0.006
HDL-C (mmol/L)	59 [45–71]	59 [49–74]	1.00
Triglycerides (mmol/L)	106 [77–137]	95 [79–121]	0.53
AST (U/L)	23 [17–27]	22 [19–26]	0.90
ALT (U/L)	14 [11–17]	12 [9–17]	0.16
γGTP (U/L)	25 [15–40]	22 [16–45]	0.92
Leukocytes (/μL)	5,100 [4,200–6,000]	5,400 [4,500–6,300]	0.68
Hemoglobin (g/dL)	12.7 [11.7–14.0]	11.3 [9.8–12.9]	0.012
eGFR (mL/min/1.73 m²)	64.5±15.4	46.9±18.2	<0.0001
PTH (intact) (pg/mL)	45 [37–61]	40 [30–69]	0.70
1,25(OH)₂VD (pg/mL)	48 [36–64]	34 [23–42]	0.0004
Calcium (mg/dL)	8.82±0.42	8.91±0.56	0.50
Inorganic phosphorus (mg/dL)	3.40±0.46	3.64±0.51	0.037
Magnesium (mg/dL)	2.26±0.19	2.20±0.23	0.15
CRP (mg/dL)	0.09 [0.03–0.21]	0.20 [0.04–0.62]	0.23
BNP (pg/mL)	59.0 [18.9–115.3]	138.6 [60.6–346.9]	0.0042
Thoracic CT			
Agatston score (HU)	655 [322–3,349]	4,082 [1,866–7,134]	0.0005
Calcification volume (HU)	955 [321–4,445]	5,248 [2,359–8,492]	0.0009
Central BP index			
Reflection magnitude (%)	67 [59–74]	65 [60–70]	0.29
Augmentation index (%)	17 [11–37]	26 [20–37]	0.09
Augmentation pressure (mmHg)	7 [4–16]	11 [6–16]	0.19
PWV (m/s)	11.4 [9.8–12.8]	12.3 [11.1–13.3]	0.040
Central SBP (mmHg)	111.1±15.8	113.6±19.0	0.53
Central DBP (mmHg)	73.5±11.1	74.3±15.3	0.80
Smoking habit			
Habitual smoker	13 (31.7)	14 (34.2)	0.84
Complications			
Hypertension	23 (56.1)	26 (63.4)	0.50
Diabetes	10 (24.4)	13 (31.7)	0.46
Hyperlipidemia	19 (46.3)	11 (26.8)	0.07

Unless indicated otherwise, data are given as the mean±SD, median [interquartile range], or n (%). Abbreviations as in Table 1.

protein (hs-CRP) concentrations, plasma BNP concentrations, Agatston scores, and calcification volume scores. Serum albumin concentrations, hemoglobin, eGFR, and vitamin D_3 were significantly lower in the higher PWV group. Systolic BP, pulse pressure, augmentation index, and augmentation pressure were significantly higher in the higher PWV group. There was no significant difference in coronary risk factors between the higher and lower PWV groups; there were relatively few patients with diabetes, obesity, or smoking history. There was also no significant difference in serum inorganic phosphorus concentrations between the 2 groups.

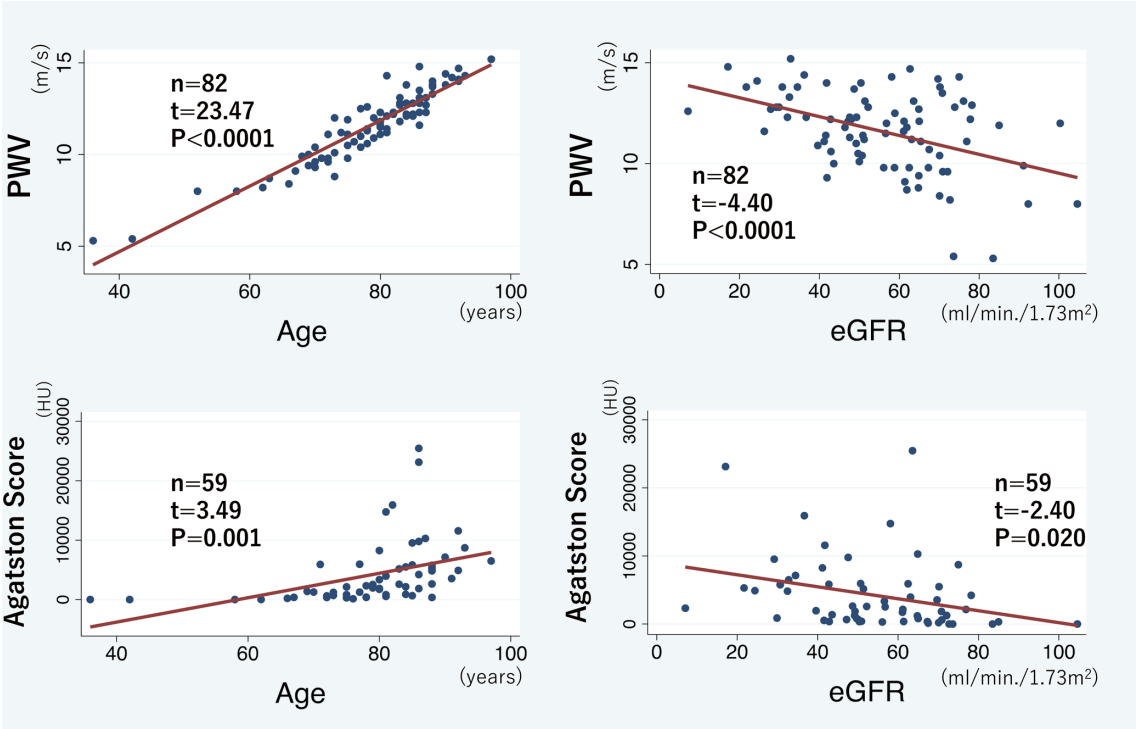

Figure 1. (**Upper**) Significant relationships between pulse wave velocity (PWV) and age and estimated glomerular filtration rate (eGFR). (**Lower**) Significant relationships between the Agatston calcification score and age and eGFR.

Clinical Characteristics in the Lower and Higher Serum FGF23 Level Groups

Table 2 presents the characteristics of patients divided into lower and higher FGF23 groups according to the medium serum FGF23 concentration (43.65 pg/mL). Compared with the lower FGF23 group, the higher FGF23 group had significantly higher age, creatinine, blood urea nitrogen, serum inorganic phosphorus concentrations, Agatston scores, calcification volume scores, and PWV. Serum albumin concentrations, total and low-density lipoprotein cholesterol, hemoglobin, eGFR, and vitamin D_3 were significantly lower in the higher FGF23 group. There was no significant difference in coronary risk factors between the 2 groups.

Concerning sex differences, there were no significant differences in age, serum concentrations of inorganic phosphorus, FGF23, vitamin D_3, intact PTH, hs-CRP, PWV, or Agatston score between males and females.

Data Analysis

Figure 1 shows significant relationships between age and both PWV and Agatston calcification score, as well as between eGFR and both PWV and Agatston calcification score. PWV had a strong relationship with age (t=23.47, P<0.0001), and a significant negative relationship with eGFR (t=−4.40, P<0.0001). Like PWV, the Agatston calcification score was also significantly related to age (t=3.49, P=0.0001) and eGFR (t=−2.40, P=0.020). There was a significant positive relationship between PWV and Agatston calcification score (t=4.36, P<0.0001).

Figure 2 shows significant relationships between serum FGF23 concentrations and both age and eGFR. Serum FGF23 concentrations increased with increasing serum inorganic phosphorus concentrations. In contrast, serum vitamin D_3 concentrations decreased significantly as serum FGF23 concentrations increased. There was a significant negative relationship between serum inorganic phosphorus and vitamin D_3 concentrations (t=−2.25, P=0.027), but not between serum inorganic phosphorus and intact PTH concentrations (t=−0.73, P=0.47). These findings indicate that the phosphaturic hormone FGF23 was activated according by an excess of phosphate and, conversely, as a positive effector of phosphate, vitamin D_3 was inhibited (**Figure 2**). There was no significant relationship between FGF23 and intact PTH (t=−1.82, P=0.07) or between vitamin D_3 and intact PTH (t=0.96, P=0.34) in this study.

Figure 3 shows that increasing serum FGF23 concentrations were positively related to both PWV and the Agatston score. Serum FGF23 concentrations were also positively related to augmentation pressure (t=2.47, P=0.015) and central systolic BP (t=2.00, P=0.049).

The ratio of serum FGF23/serum vitamin D_3 concentrations was significantly positively related to both PWV (t=3.12, P=0.003) and the Agatston score (t=2.22, P=0.031). The serum FGF23/serum vitamin D_3 concentration ratio was also significantly positively related to augmentation pressure (t=3.27, P=0.002) and central systolic BP (t=2.97, P=0.004). As shown in **Figure 4**, PWV was also significantly positively related to augmentation pressure and central systolic BP. Significant positive relationships were also found between plasma BNP concentrations and both serum FGF23 concentrations and PWV (**Figure 5**).

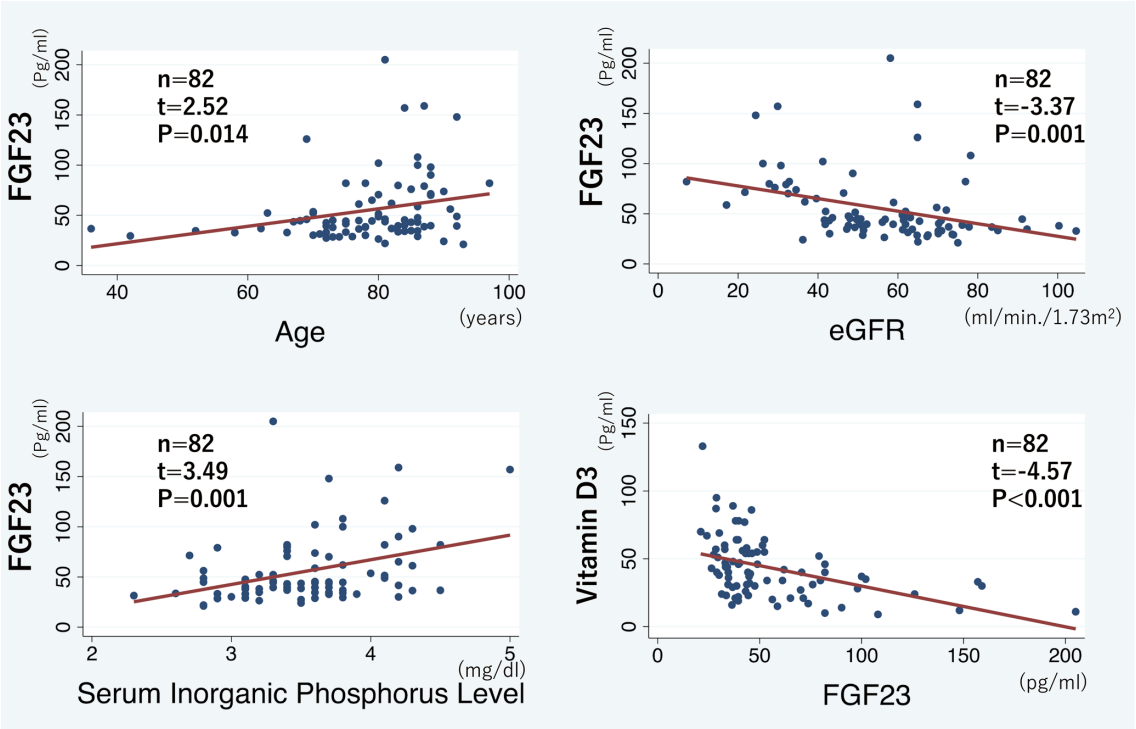

Figure 2. (**Upper**) Relationships between serum fibroblast growth factor (FGF) 23 concentrations and age and estimated glomerular filtration rate (eGFR). (**Lower**) Relationships between serum FGF23 concentrations and serum inorganic phosphorus and vitamin D_3 concentrations.

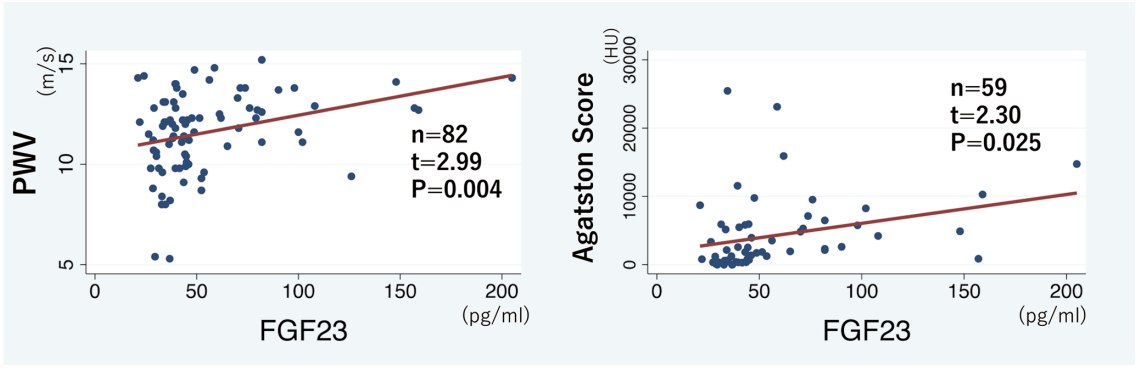

Figure 3. Relationships between the serum fibroblast growth factor (FGF) 23 concentrations and pulse wave velocity (PWV) and Agatston scores.

Multiple Logistic Regression Analysis for Arterial Stiffness: Higher PWV Group

Study subjects were divided into higher and lower PWV groups (**Table 1**). Multiple logistic regression analysis was performed using higher PWV as an independent factor. In these analysis, serum concentrations of FGF23 and hs-CRP (inflammatory marker), hypertension, and diabetes were included as dependent factors, because age and eGFR exhibited collinearity with FGF23. Serum FGF23 concentrations were significantly related to PWV (t=2.73, P=0.008), but hs-CRP (t=1.51, P=0.135), hypertension (t=1.16, P=0.248), and diabetes (t=−0.06, P=0.955) were not (**Supplementary Table**).

Discussion

This study showed that arterial stiffness was significantly correlated with age, renal dysfunction, and serum FGF23 concentrations. Furthermore serum FGF23 concentrations increased with arterial calcification and/or stiffness. We also

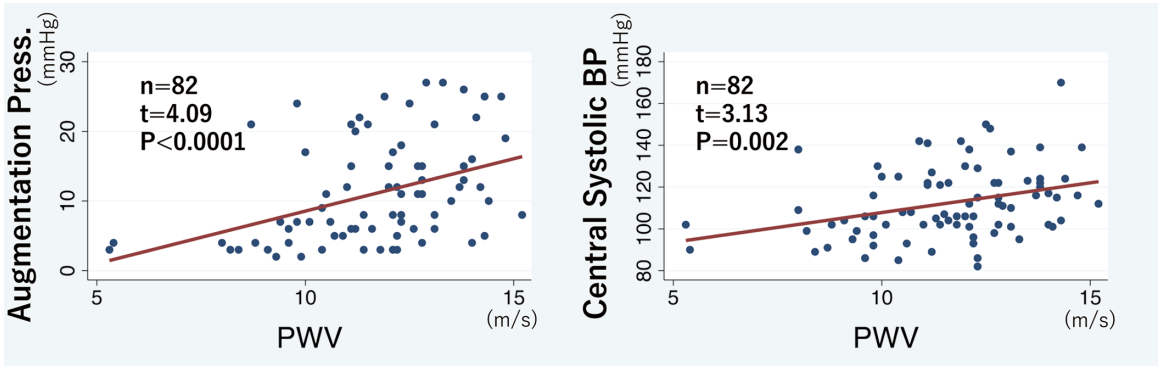

Figure 4. Relationships between pulse wave velocity (PWV) and central systolic blood pressure and augmentation pressure.

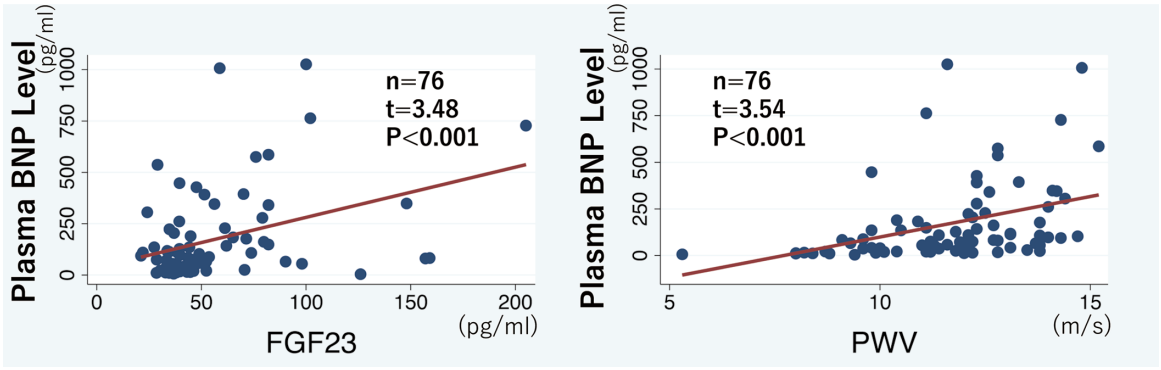

Figure 5. Positive relationships between plasma B-type natriuretic peptide (BNP) concentrations and serum fibroblast growth factor (FGF) 23 concentrations and pulse wave velocity (PWV).

showed that arterial stiffness had positive relationships with cardiac-afterload related factors, namely augmentation pressure, central systolic BP, and plasma BNP concentrations.

Attention has focused on the Klotho gene as an anti-aging factor, and the accumulation of phosphate has recently been presented as a pathogenic aging factor.[1,2,3–7] Serum phosphate concentrations are inversely correlated with average life span in mammals.[1,2,4,5] High serum phosphate concentrations are associated with high all-cause mortality.[1,2,4,5] Arterial calcification is a major risk factor for cardiovascular morbidity and mortality.[18]

The serum phosphate concentration is controlled within a narrow range by FGF23, vitamin D_3, and PTH.[1,4,5] FGF23 in particular has a central role in the management of these factors.[1,4–9]

Once the concentration of calcium and phosphate ions exceeds the blood saturation level, amorphous calcium-phosphate is precipitated and immediately absorbed by the serum protein fetuin-A to generate calcium calciprotein monomers (CPMs).[1,2,4,19,20] CPMs spontaneously aggregate to generate primary calciprotein particles (CPPs). Primary CPPs further undergo aggregation and phase transition of the calcium-phosphate form in the amorphous phase to the crystalline phase to generate secondary CPPs. Secondary CPPs induce calcification in cultured vascular smooth muscle cells, which is followed by inflammatory responses.[1,2,4,19,20] Secondary CPPs releases FGF23 mainly from the bone to decrease the excess phosphate.[1,2,4,19,20]

In this study we investigated the relationships between phosphate-related factors and arterial stiffness. It has been reported that PWV is the gold standard for measuring arterial stiffness.[21,22] In the present study, arterial stiffness was determined using PWV, and arterial calcification was estimated by arterial calcification scores on thorax CT scans. There was a significant positive relationship between arterial calcification and arterial stiffness in the present study, as reported previously.[23]

First, we found that arterial stiffness and calcification are intensified according to the degree of aging and renal dysfunction (**Figure 1**). From the relationship between age and PWV, it is easy to understand Dr. Thomas Sydenham's words, "A man is as old as his arteries".[24] As indicated in **Table 1**, the higher PWV group in the present study had increased arterial calcification-related factors (i.e., Agatston scores and calcification volume scores, systolic BP, pulse pressure, augmentation index, augmentation pressure, and serum FGF23 concentrations, and levels of inflammation), in addition to advancing age and renal dysfunction. In

addition, this group had lower serum vitamin D_3, albumin, and hemoglobin levels compared with the lower PWV group (**Table 1**). Arterial stiffness was suspected to be due to the relationship between a deterioration in phosphate homeostasis and chronic inflammation.

Serum FGF23 concentrations were also positively related to aging and renal dysfunction, both resulting in nephron loss (**Table 2**; **Figures 2**,**3**). Furthermore, serum FGF23 concentrations increased with increasing serum concentrations of inorganic phosphorus, but were negatively correlated with serum vitamin D_3 concentrations (**Figure 2**). Moreover, serum FGF23 concentrations increased with arterial calcification and/or stiffness (**Figure 3**).

Donate-Correa et al reported that patients with clinical atherosclerotic artery disease and vascular calcification have significantly higher serum FGF23 concentrations, as well as increased FGF23 protein immunoreactivity and gene expression levels in the arterial wall, compared with patients without vascular calcifications.[25] We also investigated other phosphate-regulated hormones (i.e., vitamin D_3 and PTH), as well as the relationships between artery stiffness and central BP parameters related to cardiac afterload.[21,22,26,27]

The deterioration in phosphate homeostasis is suspected to be an important cause of arterial stiffness, even in the early stages of CKD. In the present study, both a deterioration in phosphate homeostasis and arterial stiffness were associated with findings of chronic inflammation, which are characteristic of aging and non-infectious inflammatory disorders. Age and renal dysfunction were strong regulators of arterial stiffness, but these were collinear with serum FGF23 concentrations (**Figure 2**). Multiple logistic regression analysis in this study with FGF23, hs-CRP, hypertension, and diabetes as dependent factors and arterial stiffness as the independent factor revealed that for higher PWV, serum FGF23 concentration was a significant predictor for arterial stiffness.

As a key aging factor, the Klotho/FGF23 axis regulates phosphate excretion from the kidney.[1,2,4–9,19,20] Nephron loss due to aging and CKD causes problems with phosphate excretion via pituitary-specific positive transcription factors 1 and 2 on the tubules in the kidney; in addition, the intake of phosphate from processed foods, such as meat or fish bone foods, is increasing as part of our modern lifestyle to a level beyond that that can be secreted by the kidneys.[1,4–7] FGF23 accelerates urinary phosphate excretion and suppresses vitamin D_3 synthesis via restriction of 1-alpha-hydroxylase.[1,4,6,8,9] FGF23 and PTH are upregulated by phosphate accumulation.[5–9]

The most representative case of phosphate accumulation is that of hemodialysis patients.[1,2,4,5] These patients typically present severe complications of aging-related cardiovascular findings, including arterial calcification, left ventricular hypertrophy, and heart failure.[2,5–7] Phosphate accumulation can lead to a vicious cycle of accelerated aging.[1–6]

Vitamin D_3, another phosphate-regulating factor, may influence arterial calcification directly via regulation of the nuclear vitamin D receptor (VDR) or through an indirect pathway where vitamin D_3 may influence BP control by suppressing the renin-angiotensin-aldosterone system, as seen in VDR-knockout mice.[28] The major causes of vitamin D deficiency are thought to be a lack of adequate vitamin D_2 from dietary sources and avoidance of sun exposure, among others. Vitamin D deficiency and insufficiency are common problems with insidious health consequences, especially in elderly females.[10,11,28] There are some reports that vitamin D deficiency produced an aortic elastic property.[28,29] Vitamin D_3 deficiency thereby may directly produce arterial calcification and/or stiffness.[10,11,28–30] Although vitamin D_3 has favorable physiological effects, the absorption of phosphate from the intestines is unfavorable as regards phosphate control in elderly patients with CKD.[8,9] Interestingly, some studies have reported controversial results regarding the use of vitamin D_3 supplements, which led to progression of arterial calcification.[30–32] The uptake of phosphorus from the intestines by overactivation of the vitamin D_3 receptor may accelerate arterial calcification.[30–32] This means that vitamin D_3 seems to act as a double-edged sword for arterial stiffness.[32]

Finally, this study showed that a stiffer artery could produce bigger augmentation pressure and central systolic BP, which equates to cardiac afterload (**Figure 4**). Some studies have reported that a reflection of the pressure wave from stiff arteries is added on to the ejection pressure; moreover, a faster wave produces a larger loading for the left ventricle.[21,22] Augmentation pressure is the additional aortic systolic pressure produced by the return of the reflected waves at the central aorta.[21,22] Central systolic BP represents the direct pressure for the left ventricle; therefore, central systolic BP cloud be a simple and non-invasive marker of cardiac afterload. This study demonstrated that aortic stiffness, as assessed by PWV, was associated with plasma BNP concentrations. Our data are in agreement with previous reports, whereupon the stiffer the artery, the greater the amplification of cardiac afterload.[21,22,26]

The serum FGF23 concentration is a more accurate marker of phosphate homeostasis than measuring serum phosphate concentrations. Increased serum FGF23 concentrations and decreased serum vitamin D_3 concentrations could therefore be valid parameters to indicate a deterioration in phosphate homeostasis.

Perspectives

This study indicates that phosphate and its related factors could be causative of arterial stiffness. These factors play important roles in aging-related diseases, including cardiovascular diseases. Phosphate treatments using FGF23 and soluble Klotho, as well as vitamin D_3-modulating treatment, will be advantageous as new therapies. We theorize that arterial stiffness is an important target in many cardiovascular diseases.

Study Limitations

This was a cross-sectional study with a limited number of patients. In addition, the number of patients with CT tests was limited due to concerns regarding radiation exposure. We are not able to present a cause-effect relationship for arterial stiffness.

Conclusions

Arterial calcification and/or stiffness increase in parallel with aging and renal dysfunction, due to nephron loss, which, in turn, results in an excess of phosphate. The deterioration in phosphate homeostasis is an important trigger for accelerated aging. Increasing serum FGF23 concentrations may be a clinically an important predictor for arterial calcification and/or stiffness, which increases cardiac afterload.

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Disclosures

The authors declare no conflicts of interest.

Author Contributions

Y.M., T.I., F.K., S.T., K.Y., and E.H. conceived and designed the study and collected the data. Y.M. and Y.N. performed the analysis. Y.M. wrote the paper. Y.M., Y.N., and E.H. critically reviewed the manuscript.

IRB Information

This study was approved by the Ethics Committee of our institution (Approval no. JMC 269-2108).

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Supplementary Files

Please find supplementary file(s);
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