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Arterial Stiffness Is an Important Predictor of Heart Failure with Preserved Ejection Fraction (HFpEF)—The Effects of Phosphate Retention

Yuji Mizuno [1,2,*], Toshifumi Ishida [1,3], Kenichi Tsujita [3] and Michihiro Yoshimura [4]

[1] Division of Cardiovascular Medicine, Kumamoto Aging Research Institute, Kumamoto 860-8518, Japan; ishida-t@kumamoto-u.ac.jp
[2] Division of Cardiovascular Medicine, Mizuno Heart Clinic, Koshi 861-1115, Japan
[3] Department of Cardiovascular Medicine, Faculty of Life Science, Graduate School of Medical Sciences, Kumamoto University, Kumamoto 860-8556, Japan; tsujita@kumamoto-u.ac.jp
[4] Division of Cardiology, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo 105-0003, Japan; m.yoshimura@jikei.ac.jp
* Correspondence: aldh2@icloud.com; Tel.: +81-96-248-3355

Abstract: Heart failure with preserved ejection fraction (HFpEF) is a major health concern. There is a growing recognition of the causal interplay between arterial stiffness and HFpEF. We recently reported that phosphate retention is a trigger for arterial stiffness. This study focuses on whether arterial stiffness due to phosphate retention could be a predictor for HFpEF. Methods: The subjects of this study were 158 patients (68 males and 90 females, mean age 74.8 ± 11.2). HFpEF was defined according to the guidelines of the ESC 2019. Pulse wave velocity (PWV) and central systolic blood pressure (CSBP) were used as markers for arterial stiffness and afterload, respectively. We measured serum levels of fibroblast growth factor 23 (FGF23) as a marker of phosphate retention. Results: The serum levels of FGF23 had a significant relationship with PWV. PWV had significant relationships with LV mass index, plasma BNP levels, and relative wall thickness, e′, and E/e′ ($p < 0.001$, respectively). Multivariate logistic regression analysis revealed that higher PWV values and hypertension were significant predictors for the dependent factor (HFpEF). Arterial stiffness amplified afterload, leading to LV concentric hypertrophy and diastolic dysfunction. This study presents that arterial stiffness is a key predictor of HFpEF, and that phosphate retention is involved in the pathology of HFpEF.

Keywords: HFpEF; arterial stiffness; phosphate; FGF23; VD_3; PWV; afterload; BNP

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1. Introduction

Heart failure with preserved ejection fraction (HFpEF) is a worldwide health concern. The number of patients with HFpEF is increasing as populations age. However, the mechanisms underlying HFpEF are unclear [1–4]. There is a growing recognition of the causal interplay between arterial stiffness and diastolic dysfunction and/or HFpEF [5–10]. It is not widely known what the main cause of arterial stiffness is, nor how it leads to the pathological mechanism of HFpEF.

Hemodialysis patients present the most representative model of HFpEF [7–9], as they present the following characteristics: renal dysfunction; arterial calcification; left ventricular (LV) concentric hypertrophy; LV diastolic dysfunction; hypertension; osteoporosis; and accelerated aging [7,9,11].

The Klotho/Fibroblast growth factor 23 (FGF23) axis has been focused on as an important regulating factor of accelerated aging, including cardiovascular mortality [8,12–15]. In response to phosphate intake, FGF23 is secreted mainly from the bones, circulates in the blood, and binds to the Klotho-FGF receptor complexes expressed in the kidneys to

promote urinary phosphate excretion [8,13–15]. The FGF23-Klotho endocrine system is indispensable for maintaining phosphate homeostasis. Loss-of-function mutations in either FGF23 or Klotho cause phosphate retention phenotypes including ectopic calcification and hyperphosphatemia [8,13–15]. The serum phosphate level is controlled within a small range, whereas phosphate homeostasis is maintained by a counterbalance between the absorption of dietary phosphate from the intestines and the excretion of phosphate from the blood via the kidneys into the urine [8,13–15].

Parathyroid hormone (PTH) is also a phosphaturic hormone [13,15]. Like FGF23, PTH exerts phosphaturic activity by suppressing phosphate reabsorption at the renal tubules [16,17]. In the parathyroid organ, FGF23 suppresses production and secretion of PTH, whereas PTH reciprocally induces FGF23 expression [16,17].

Vitamin D3 (VD3) is a physiologically important active hormone against atherosclerosis, endothelial dysfunction, inflammation, osteoporosis, and LV hypertrophy [18,19]. VD3 is activated by the kidneys and acts on the intestines to increase the absorption of phosphate and calcium, thereby inducing a positive phosphate balance [16–19]. VD3 is, however, inhibited by FGF23 in a state wherein the retention of phosphate is in balance [16,17]. It is therefore agreed that FGF23 is the most potent hormone regulating phosphate homeostasis and phosphate retention [13,15–17]. Phosphate retention and phosphate-regulating hormones could therefore be responsible for pathological arterial calcification [13,15–17].

We recently reported that arterial calcification and/or stiffness increases in parallel with aging and renal dysfunction due to nephron loss, which in turn results in phosphate retention. The deterioration of phosphate homeostasis is an important trigger for arterial stiffness, by measuring of FGF23 [20]. It has not yet been understood how arterial stiffness leads to the pathology of HFpEF. To understand the pathological mechanism of HFpEF, we investigated whether arterial stiffness may be affected by phosphate retention, and whether this could be a predictor for HFpEF, by using logistic regression analysis.

2. Materials and Methods

2.1. Study Subjects

This was a prospective cross-sectional study comprising 158 consecutive Japanese patients with Table 1, New York Heart Association Classification II–III, 68 males and 90 females, with a mean age of 74.8 ($\pm$11.2) who were admitted at, or referred to, our institution or outpatient clinic between May 2018 and March 2022. All subjects presented, or had presented, with the following: (1) Symptoms (dyspnea, fatigue, and fluid retention) and signs of heart failure; (2) LV ejection fraction (LVEF) $\geq$ 50%. The subjects were divided into an HFpEF group and a non-HFpEF group according to the guidelines of 2019 ESC HFpEF scores. Scores were calculated based on objective evidence of cardiac structural and/or functional abnormalities consistent with the presence of LV diastolic dysfunction, such as raised LV filling pressures, with each of the following items constituting one point: (1) LV mass index 115 $\geq$ (male) or $\geq$95 g/m^2 (female); (2) Relative wall thickness > 0.42; (3) Early diastolic mitral flow velocity (E)/tissue annular motion velocity (e′) or E/e′ $\geq$ 15 on echocardiogram; (4) Elevated plasma BNP levels $\geq$ 35 pg/mL in sinus rhythm (SR) and $\geq$105 pg/mL in atrial fibrillation (AF); and (5) Pulmonary systolic pressure > 35 mmHg or TR velocity > 2.8 m/s, with reference to the guidelines of ESC [4]. Participants were divided into two groups depending on whether their total score exceeded 5 points. Patients with acute decompensated heart failure (LVEF < 50%), acute myocardial infarction, acute inflammatory disease, pericardial disease, severe valvular heart disease, congenital heart disease, cardiomyopathies, and hemodialysis patients were excluded from this study. The main diseases in the non-HFpEF group were ischemic heart disease in 45 cases, chest pain syndrome in 40 cases, chronic obstructive pulmonary disease in 15 cases, and other diseases in 6 cases. This study was conducted in accordance with the Declaration of Helsinki and approved by the ethical committee of our institution (approval Juryo Medical Corporation No. 269-2108, Kumamoto Kinoh Hospital Ethical Review Committee, Yamamuro 6-8-1, Kitaku, Kumamoto city, Japan).

Table 1. Comparison between HFpEF group and non-HFpEF group.

Variables	HFpEF Group (n = 52)	Non-HFpEF Group (n = 106)	*p* Value
Age, years	82.0 ± 6.6	71.3 ± 11.4	<0.0001
Sex, male, n (%)	16 (30.8%)	52 (49.1%)	0.029
Body mass index, kg/m^2	23.5 ± 4.2	23.1 ± 3.8	0.51
Systolic blood pressure, mmHg	132.0 ± 16.8	125.1 ± 18.2	0.024
Diastolic blood pressure, mmHg	68.4 ± 12.4	71.3 ± 11.2	0.14
Heart rate, beats/min	73.9 ± 15.9	73.0 ± 14.7	0.72
Pulse pressure, mmHg	63.6 ± 14.6	53.7 ± 14.7	<0.001
-Blood tests			
Albumin, g/dL	3.6 ± 0.6	4.0 ± 0.4	<0.0001
Glucose, mg/dL	103 (87, 113)	106 (94,127)	0.07
Hemoglobin A1c, %	5.9 (5.6, 6.4)	6.0 (5.6, 6.7)	0.46
Creatinine, mg/dL	1.1 ± 0.8	0.9 ± 0.3	0.014
BUN, mg/dL	22.3 ± 10.7	18.5 ± 8.9	<0.001
eGFR, mL/min/1.73 m^2	51.9 ± 17.1	62.5 ± 19.3	<0.001
Total cholesterol, mg/dL	187 (148, 215)	187 (163, 210)	0.67
LDL-cholesterol, mg/dL	91 (72, 117)	99 (76, 120)	0.64
HDL-cholesterol, mg/dL	63 (45, 82)	61 (50, 75)	0.56
Triglycerides, mg/dL	100 (79, 134)	112 (85, 159)	0.17
AST, U/L	22 (19, 29)	22 (18, 27)	0.38
ALT, U/L	14 (10, 20)	14 (10, 22)	0.33
γGTP, U/L	22 (15, 40)	26 (17, 45)	0.40
LDH, U/L	225 (173, 275)	166 (150, 184)	<0.0001
ALP, U/L	181 (90, 246)	127 (72, 223)	0.14
hs-CRP, mg/dL	0.21 (0.06, 0.61)	0.08 (0.04, 0.19)	0.007
Leukocyte,/μL	5350 (4100, 6100)	5500 (4500, 6400)	0.29
Hemoglobin, g/dL	11.4 ± 1.9	13.5 ± 1.8	<0.0001
Platelets, ×10^4/μL	20.3 ± 7.8	21.3 ± 6.4	0.42
PTH (intact), pg/mL	50.0 (35.0, 70.5)	39.0 (29.0, 55.0)	0.07
1,25(OH)2VD, (VD3), pg/mL	34.0 (27.0, 46.0)	48.0 (37.0, 59.0)	<0.010
FGF23, pg/mL	51.2 (34.7, 79.8)	42.6 (34.6, 48.7)	0.042
Calcium, mg/dL	8.8 ± 0.5	9.0 ± 0.5	0.07
Phosphate, mg/dL	3.51 ± 0.52	3.51 ± 0.46	0.97
Magnesium, mg/dL	2.22 ± 0.28	2.23 ± 0.17	0.96
BNP, pg/mL	145.4 (73.5, 343.2)	26.7 (13.4, 60.1)	<0.0001
Sinus	77.9 (60.9, 126.1)	23.7 (12.7, 50.9)	<0.0001
Af	267.0 (145.5, 491.9)	50.1 (36.8, 176.9)	<0.0001
-Thoraxic CT (Calcification)			
Thoraxic Agatston score, HU	4182 (874, 9532)	1372 (322, 4213)	0.003
Calcification volume score, HU	5666 (1291, 11,479)	1930 (436, 5669)	0.004
-Central blood pressure index			
Reflection magnitude, %	65 (60, 72)	67 (59, 74)	0.60
Augmentation press., mmHg	10 (6, 13)	7 (4, 15)	0.039
Augmentation index, %	25 (19, 33)	20 (11, 37)	0.029
PWV, m/s	12.3 (10.9, 13.1)	10.2 (9.1, 11.8)	<0.0001
Central SBP, mmHg	121.1 ± 16.6	110.0 ± 16.5	<0.001
Central DBP, mmHg	77.3 ± 14.8	75.6 ± 12.5	0.46
Central pulse pressure, mmHg	43.7 ± 13.5	34.5 ± 10.3	<0.0001
-Cardiac echo			
LVDd, mm	43.0 ± 5.5	42.0 ± 5.2	0.27
LVDs, mm	27.2 ± 4.8	26.5 ± 4.9	0.40
IVST, mm	10.5 ± 1.6	9.8 ± 1.5	0.006
PWT, mm	10.3 ± 1.7	9.5 ± 1.5	0.005
LVMI, g/m^2	102 (80, 118)	81 (69, 96)	<0.0001
Relative wall thickness	0.46 (0.41, 0.55)	0.42 (0.40, 0.46)	0.002
Septal e′	5.2 (4.3, 6.0)	7.2 (6.1, 9.0)	<0.0001
Septal E/e′ ratio	16.0 (15.3, 17.6)	10.0 (8.1, 11.6)	<0.0001
TR velocity, m/sec	2.7 (2.6, 3.0)	2.4 (2.2, 2.6)	<0.0001

Table 1. *Cont.*

Variables	HFpEF Group (n = 52)	Non-HFpEF Group (n = 106)	*p* Value
LVEF (teichholz), %	67.3 (61.0, 72.6)	68.4 (63.0, 72.7)	0.53
E/A ratio,	0.81 ± 0.37	0.77 ± 0.25	0.49
Dct time, mmsec	221.8 ± 90.3	226.5 ± 56.0	0.69
LA dimension, mm	41.4 ± 8.6	34.0 ± 6.4	<0.0001
-Smoking habit			
Habitual smoking, n (%)	12/41 (29.3%)	37/82 (45.1%)	0.09
-Complications			
Hypertension, n (%)	39 (75.0%)	61(57.5%)	0.032
Diabetes mellitus, n (%)	13 (28.8%)	24 (22.6%)	0.40
Hyperlipidemia, n (%)	10 (19.2%)	26 (24.5%)	0.46
Af history, n (%)	28 (53.8%)	21 (19.8%)	<0.0001

Af indicates atrial fibrillation; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BNP: B type natriuretic peptide; BUN: blood urea nitrogen; CKD: chronic kidney disease; DBP: diastolic blood pressure; Dct: deceleration time; e-GFR: estimated glomerular filtration rate; FGF23: fibroblast growth factor 23, HDL: high-density lipoprotein; HFpEF: heart failure with preserved ejection fraction; hs-CRP: high sensitivity C-reactive protein; HU: hounsfield units; IVST: interventricular septal thickness; LA: left atrial; LDL: low-density lipoprotein; LVDd: left ventricular diastolic dimension; LVDs: left ventricular systolic dimension; LVEF: left ventricular ejection fraction; LVMI: left ventricular mass index; press.: pressure; PWT: posterior wall thickness; PTH: parathyroid hormone, PWV: pulse wave velocity; SBP: systolic blood pressure; γGTP: γ-Glutamyl Trans Peptidase; 1,25(OH)2VD: 1-25 dihydroxy vitamin D3.

2.2. Echocardiography

Echocardiography, including two-dimensional, pulse, and continuous wave Doppler, color flow Doppler, and tissue Doppler imaging were performed using the iE33 Ultrasound System (Philips Ultrasound Co., Bothell, Washington, DC, USA) with the patients stable at the time of examination, either as an ambulatory outpatient or inpatient on the same day, or within 2 days of blood sampling for BNP levels. LV and atrial linear dimensions were measured from two-dimensional echocardiographic images, and peak E-wave and A-wave velocity, E/A, E/e′, LV diastolic dimension (LVDd), LV systolic dimension (LVDs), LV mass index (LVMI), left atrial dimension (LAD), right ventricular dimension (RVD), pulmonary artery systolic pressure (PASP), interventricular-septal thickness (IVST), posterior-wall thickness (PWT), relative wall thickness (RWT), stroke volume (SV), and LVEF were measured and calculated according to the recommendations of the American Society of Echocardiography and the European Association of Echocardiography [21]. LV mass was estimated using linear measurements from two-dimensional images and indexed to body surface area as LVMI. LV geometry was classified based on RWT, defined as (2 × diastolic posterior wall thickness)/LVDd), and LVMI was classified as follows: normal, RWT $\leq$ 0.42 and no LV hypertrophy (LVH); eccentric hypertrophy, RWT $\leq$ 0.42 and LVH; concentric remodeling, RWT > 0.42 and no LVH; or concentric hypertrophy, RWT > 0.42 and LVH [21]. Echocardiography was performed by experienced sonographers who were unaware of the clinical information of each patient.

2.3. Assessments of Pulse Wave Velocity and Central Blood Pressure Measurements

This study used pulse wave velocity (PWV) as a marker of arterial stiffness [10,22]. PWV and central blood pressure-related parameters were detected through the Mobil-O-Graph pulse wave analysis (PWA)/ambulatory BP monitoring device (I.E.M. GmBH, Stolberg, Germany), which performs a cuff-based oscillometric method of measurement. The device was approved for blood pressure measurement by the British Hypertension Society and the European Society of Hypertension, and device reliability was demonstrated in comparisons through invasive and non-invasive methods for PWA [23,24]. Blood pressure (BP) measurements were performed on patients' left upper arms in a sitting position after a 10 min rest, with the patients' left elbows flexed and supported at the heart level on the chair. Augmentation pressure, augmentation index (AI), and central BP, including general brachial artery BP measurements, were measured [23,24].

Blood pressure: brachial BP was measured with an Omuron HEM 705-CP semiautomatic oscillometric recorder, using the mean of three BP values in the echocardiographic laboratory. Pulse pressure (PP) was calculated as systolic BP minus diastolic BP.

2.4. Thoracic Aortic Calcification (TAC) Scores

TAC burden was measured from each participant's computed tomographic scan, and TAC was taken from the aortic annulus, above the aortic valve, to the lower edge of the pulmonary artery bifurcation (ascending aorta), and from the lower edge of the pulmonary bifurcation to the cardiac apex (descending aorta). The TAC score was determined for each study participant using the Agatston score and calcification volume score, which have been widely used in the scientific literature as a convenient TAC quantification method [25].

2.5. Blood Chemistry Measurements

Blood samples for measurement of clinical chemistry and other data were collected from the 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 time of assay.

Serum FGF23 levels were measured by an enzyme-linked immunosorbent assay (ELISA) that recognized only full-length biologically active FGF23 with a detection limit of 3 pg/mL (Kainos, Japan). The reference range of FGF23 in healthy adults measured by this ELISA was 10–50 pg/mL, with a mean value of about 30 pg/mL [26].

Levels of an active form of Vitamin D, 1,25(OH)2D: (VD3) were determined at baseline with a fully automated and sensitive immunoassay that used a recombinant fusion construct of the vitamin D receptor ligand binding domain for the specific capture of VD3 (DiaSorin, Saluggia, Italy). The limit of quantification for this VD3 assay was 5 pg/mL, and the reference interval determined in healthy volunteers ranged between 25.0 and 86.5 pg/mL, with a median of 48.1 pg/mL [27].

The intact PTH assay was performed using Allegro Intact PTH (I-Nichols, San Juan Capistrano, CA, USA). Normal values ranged from 10 to 65 pg/mL [28].

Plasma BNP levels were measured using a specific immunoradiometric assay for human BNP (TOSOH Corp, Tokyo, Japan) [29]. The minimal detectable quantity of human BNP was 2.0 pg/mL. The mean intra-assay and inter-assay coefficients of variation were 2.3% and 3.0%, respectively.

2.6. Bone Mineral Density (BMD)

BMD was measured by dual-energy X-ray absorptiometry using Delphi W (Hologic company, Marlborough, MA, USA).

2.7. Statistical Analysis

The baseline clinical data were expressed as the mean ± SD or median (25th, 75th percentile) for continuous variables, and differences within the group were evaluated with the unpaired *t*-test or the Mann–Whitney rank sum test. For discrete variables, the data were expressed as counts and percentages and analyzed with the Chi square test. Classification of habitual smoking included current and past smokers. Linear regression analysis was used to assess the association between each parameter. PWV levels were divided by the median of each parameter for logistic analysis. Collinearity was estimated in the selection of independent variables for the dependent variable in multivariable logistic analyses. A two-tailed value of $p < 0.05$ was considered to be statistically significant. The analyses were performed using the STATA software program (STATA 18.0, STATA Corp., College Station, TX, USA).

3. Results

3.1. Clinical Characteristics

Table 1 compares the clinical characteristics between the HFpEF group and the non-HFpEF group. The HFpEF group exhibited significant increases in the following properties: age; female rate; systolic BP; PP; plasma level of creatinine; blood urea nitrogen (BUN); lactate dehydrogenase (LDH); hypersensitive C-reactive protein (hs-CRP); FGF23; and BNP. The HFpEF group also had significantly lower levels of estimated glomerular filtration rate (eGFR) and plasma levels of albumin, hemoglobin, and VD3 compared with the non-HFpEF group. The HFpEF group indicated significantly higher aortic calcification scores, Agatston score, and calcium volume score compared with the non-HFpEF group. The HFpEF group moreover presented higher levels of arterial stiffness-related parameters, including AI, PWV, systolic BP, PP, central systolic BP, and central PP. Furthermore, the HFpEF group had a higher level of LV thickness, IVST, PWT, RWT, and LV diastolic dysfunction markers, including E/e′, e′, and LAD. They frequently were receiving hypertension and heart failure medications, and they had a history of AF compared with the non-HFpEF group. There were no differences regarding the characteristics of obesity and/or DM in this study.

3.2. Correlations between Each Parameter

Age had a significant positive relationship with PWV, and eGFR had a significant negative relationship with PWV (Figure 1).

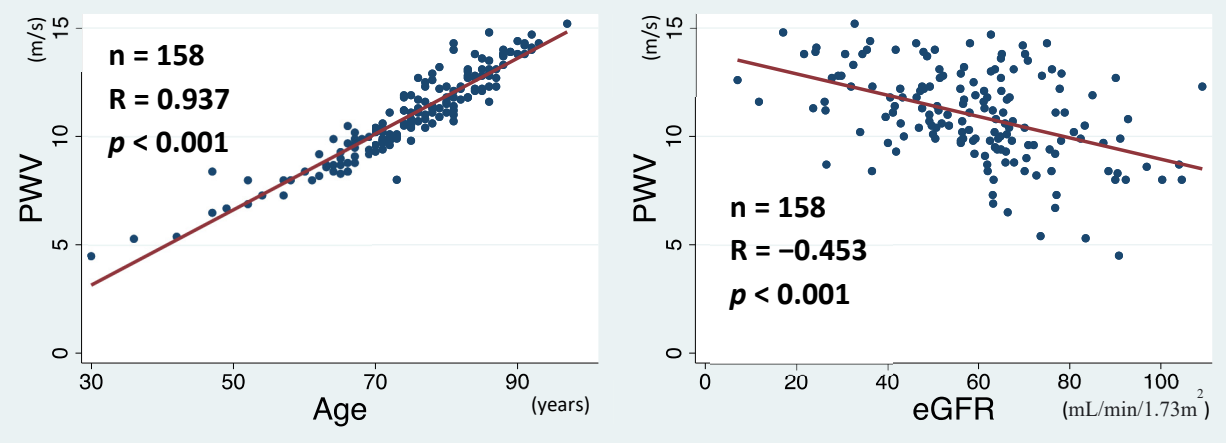

Figure 1. The relationships between PWV and age, as well as eGFR. PWV, an arterial stiffness marker, shows significant relationships with age and eGFR.

The serum levels of FGF23 had a significant positive relationship with PWV (r = 0.339, $p = 0.001$), and that of VD3 had a significant negative relationship with PWV (r = −0.219, $p = 0.024$).

There were significant positive correlations between PWV and augmentation pressure, as well as central systolic BP (Figure 2).

PWV had a significant positive correlation with LV hypertrophic markers, such as LVMI and plasma levels of BNP. Additionally, it showed a significant positive correlation with RWT, or so-called concentric hypertrophy (Figure 3).

Finally, PWV had significant correlations with e′ and E/e′ ratio (Figure 4).

Central systolic BP, which is an indicator of LV afterload, had a significant relationship with LVMI (r = 0.243, $p = 0.002$), e′ (r = −0.290, $p < 0.001$), and E/e′ (r = 0.199, $p = 0.012$).

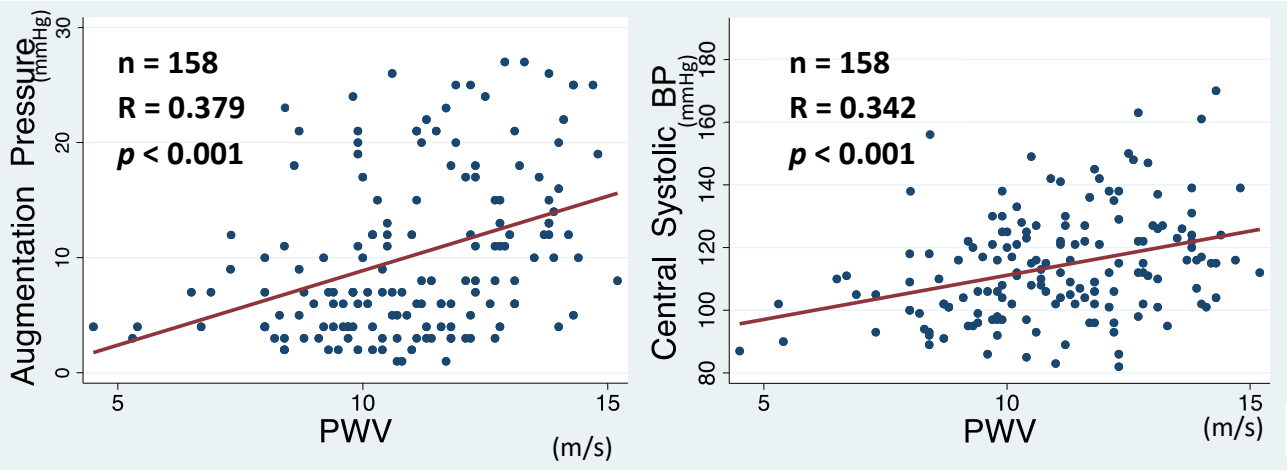

Figure 2. The relationships between PWV and afterload. PWV shows a significant positive relationship with augmentation pressure and central systolic blood pressure, which is an indicator of LV afterload.

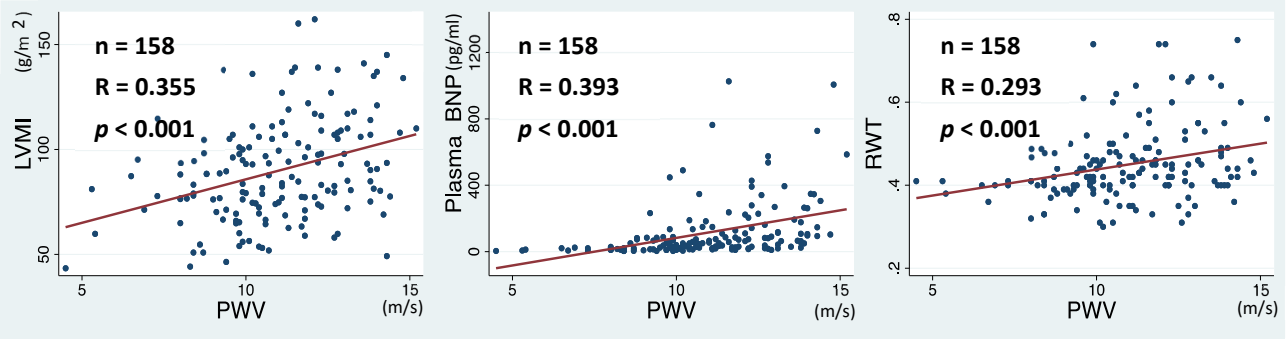

Figure 3. The relationships between PWV and LV hypertrophic markers. PWV shows significant positive relationships with LV mass index (LVMI) and plasma levels of BNP. PWV has a positive relationship with relative wall thickness (RWT), a marker of concentric hypertrophic hypertrophy.

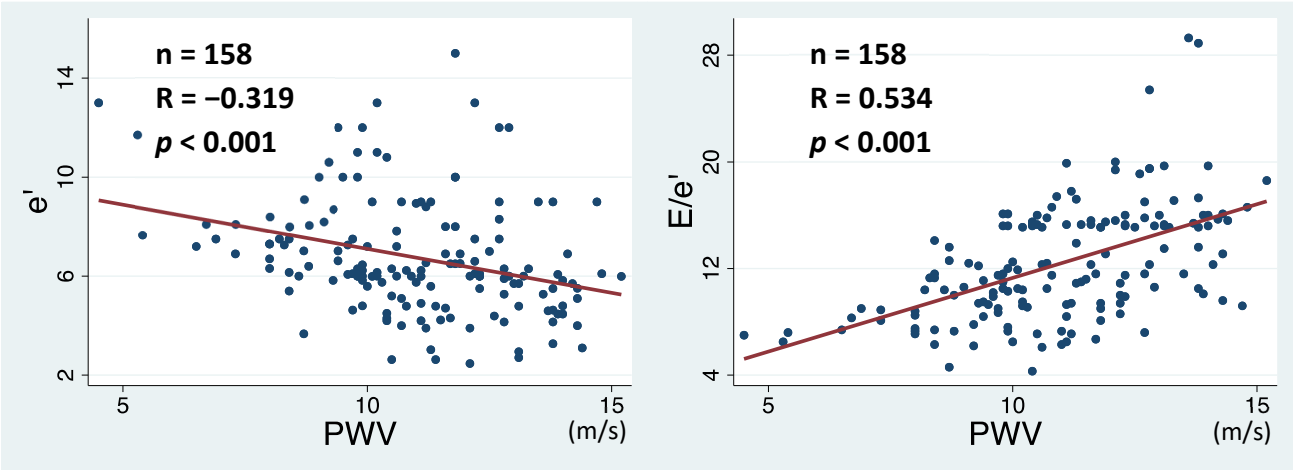

Figure 4. The relationships between PWV and HFpEF judgement parameters. PWV has significant relationships with e′ and E/e′, which are judgement parameters of HFpEF.

Females had significantly higher values in age (76.7 ± 9.6 vs. 72.4 ± 12.6 years, $p = 0.017$), serum phosphate level (3.60 ± 0.50 vs. 3.36 ± 0.44 mg/dL, $p = 0.016$), central pulse pressure (40.7 ± 12.7 vs. 33.5 ± 10.1 mm Hg, $p < 0.001$), AI (25 (17, 38) vs. 17 (9, 30) %, $p = 0.0016$), and E/e′ (11.6 (8.5, 15.8) vs. 9.0 (6.5, 13.3), $p = 0.0136$). Females had significantly lower values in BMI (22.7 ± 4.4 vs. 24.0 ± 3.1 kg/m^2, $p = 0.044$), hemoglobin (12.2 ± 1.55 vs. 13.6 ± 2.41 g/dL, $p < 0.001$), glucose (99 (91, 113) vs. 111 (99, 129) mg/dL, $p = 0.003$), γGTP (20 (14, 28) vs. 39 (28, 61) IU/L, $p < 0.0001$), and hip bone mineral density (0.69 (0.53, 0.79) vs. 0.85 (0.72, 1.01) g/cm^2, $p < 0.001$) (Table 2).

Table 2. Comparison between males and females.

Variables	Male (n = 68)	Female (n = 90)	*p* Value
Age, years	72.4 ± 12.8	76.7 ± 9.6	0.017
Body mass index, kg/m^2	24.0 ± 3.1	22.7 ± 3.1	0.044
Systolic blood pressure, mmHg	127.0 ± 18.9	127.7 ± 17.7	0.81
Diastolic blood pressure, mmHg	71.8 ± 12.1	69.2 ± 11.2	0.18
Heart rate, beats/min	71.1 ± 14.0	74.8 ± 15.6	0.14
Pulse pressure, mmHg	55.2 ± 15.5	58.4 ± 15.2	0.19
-Blood tests			
Albumin, g/dL	3.9 ± 0.5	3.8 ± 0.5	0.30
Glucose, mmol/L	110 (97, 129)	99 (91,113)	0.006
Hemoglobin A1c, %	6.2 (5.6, 6.9)	5.9 (5.6, 6.1)	0.10
Creatinine, mg/dL	1.1 ± 0.5	0.9 ± 0.5	0.024
BUN, mg/dL	18.5 ± 8.8	20.7 ± 10.2	0.15
eGFR,	59.8 ± 16.4	58.5 ± 21.1	0.68
Total cholesterol, mmol/L	172 (149, 198)	199 (166, 215)	0.002
LDL-cholesterol, mmol/L	93 (68, 113)	101 (82, 123)	0.11
HDL-cholesterol, mmol/L	53 (41, 67)	69 (59, 82)	<0.0001
Triglycerides, mmol/L	111 (99, 144)	104 (77, 143)	0.35
AST, U/L	23 (19, 29)	22 (18, 26)	0.31
ALT, U/L	17 (13, 26)	12 (9, 17)	<0.001
γGTP, U/L	39 (28, 61)	20 (14, 28)	<0.0001
LDH, U/L	174 (150, 214)	181 (159, 215)	0.29
ALP, U/L	145 (70, 242)	166 (89, 245)	0.16
hs-CRP, mg/dL	0.14 (0.06, 0.35)	0.09 (0.03, 0.22)	0.042
Leukocyte,/μL	5500 (4400, 6500)	5400 (4400, 6100)	0.35
Hemoglobin, g/dL	13.6 ± 2.4	12.2 ± 1.5	<0.0001
Platelets, ×10^4/μl	20.0 ± 6.4	21.7 ± 7.2	0.15
PTH (intact), pg/mL	41.5 (30.0, 56.0)	40.0 (31.0, 64.0)	0.81
1,25(OH)2VD, (VD3), pg/mL	41.0 (30.0, 57.0)	44.0 (30.0, 58.0)	0.91
FGF23, pg/mL	42.9 (37.5, 52.3)	45.0 (33.6, 73.8)	0.42
Calcium, mg/dL	8.8 ± 0.4	9.0 ± 0.5	0.08
Phosphate, mg/dL	3.36 ± 0.44	3.60 ± 0.50	0.016
Magnesium, mg/dL	2.23 ± 0.17	2.23 ± 0.24	0.97
BNP, pg/mL	38.2 (19.7, 101.3)	60.9 (19.0, 134.9)	0.27
Sinus	24.7 (14.2, 60.1)	34.9 (14.4, 85.0)	0.23
Af	175.4 (50.7, 345.6)	176.9 (63.4, 391.5)	0.63
-Thoraxic CT (Calcification)			
Thoraxic Agatston score, HU	3333 (1207, 8622)	1956 (369, 5486)	0.26
Calcification volume score, HU	4558 (1093, 8622)	2543 (550, 7174)	0.21
-Central blood pressure index			
Reflection magnitude, %	65 (59, 70)	69 (61, 75)	0.025
Augmentation press., mmHg	7.0 (4.0, 12.5)	8.0 (5.0, 17.0)	0.032
Augmentation index, %	17.0 (9.0, 30.0)	24.5 (17.0, 38.0)	0.001
PWV, m/s	10.5 (9.5, 12.5)	11.2 (9.9, 12.6)	0.12
Central SBP, mmHg	112.7 ± 17.4	112.7 ± 17.0	0.47
Central DBP, mmHg	78.9 ± 12.8	74.0 ± 13.0	0.018
Central pulse pressure, mmHg	55.2 ± 15.5	58.4 ± 15.2	0.19

Table 2. *Cont.*

Variables	Male (n = 68)	Female (n = 90)	*p* Value
-Cardiac echo			
LVDd, mm	45.0 ± 4.2	40.3 ± 5.2	<0.0001
LVDs, mm	29.2 ± 4.5	25.0 ± 4.3	<0.0001
IVST, mm	10.3 ± 1.5	9.8 ± 1.6	0.023
PWT, mm	10.2 ± 1.6	9.5 ± 1.5	0.006
LVMI, g/m^2	93.5 (77.9, 107.0)	80.5 (71.3, 100.0)	0.028
Relative wall thickness	0.42 (0.40, 0.47)	0.44 (0.40, 0.49)	0.11
Septal e′	6.5 (5.8, 9.0)	6.0 (4.8, 7.3)	0.006
Septal E/e′ ratio	10.8 (8.0, 15.2)	12.4 (9.6, 15.5)	0.029
TR velocity	2.5 (2.3, 2.9)	2.6 (2.3, 2.8)	<0.0001
LVEF (teichholz), %	65.9 (59.3, 71.5)	69.1 (64.0, 74.7)	0.53
E/A ratio,	0.83 ± 0.34	0.75 ± 0.23	0.14
Dct time, mmsec	215.4 ± 66.8	232.1 ± 70.1	0.13
LA dimension, mm	37.5 ± 8.0	35.6 ± 7.9	0.14
-Smoking habit			
Habitual smoking, n (%)	41 (60.3 %)	8 (8.9 %)	<0.0001
-Complications			
HFpEF, n (%)	16 (23.5 %)	36 (40.0 %)	0.029
Hypertension, n (%)	38 (55.9 %)	62 (68.9 %)	0.09
Diabetes mellitus, n (%)	23 (33.8 %)	16 (17.8 %)	0.021
Hyperlipidemia, n (%)	11 (16.2%)	25 (27.8 %)	0.09
Af history, n (%)	22 (32.4 %)	27 (30.0 %)	0.75
Osteoporosis, n (%)	7/24 (29.2%)	33/52 (63.5%)	0.005
Hip total BMD, 0.75(YAM80%)	87.2 ± 20.1	68.2 ± 17.1	<0.0001

Abbreviations are the same as shown in Table 1.

The rate of diabetes was significantly higher in males than in females (23/68 (33.8%) vs. 16/90 (17.8%), $p = 0.021$). DM patients presented significantly increased aortic calcification scores compared with non-DM patients: Agatston score (3889 (1911, 8031) vs. (1412 (369, 5771) HU, $p = 0.040$) and aortic calcium volume score (5883 (2795, 10,729) vs. (2047 (550, 7195) HU, $p = 0.021$), although there was no significant difference in PWV (11.2 (10.2, 12.5) vs. 10.9 (9.4, 12.7), $p = 0.31$, respectively) in this study.

3.3. Regression Analyses

The results of single regression analyses for HFpEF are shown in Table 3.

Table 3. Single linear regression analyses for HFpEF.

HFpEF	Coef.	Std. Err.	t	$p > \|t\|$	95% Conf. Interval	
PWV	0.11133	0.01573	7.08	<0.001	0.0803	0.1424
Age	0.01871	0.00300	6.23	<0.001	0.0128	0.0246
Central systolic BP	0.00822	0.00210	3.92	<0.001	0.0041	0.0124
eGFR	−0.00713	0.00188	−3.79	<0.001	−0.0108	−0.0034
Agatston score	0.00003	0.00001	2.98	0.004	0.0000	0.0001
Sex (male)	−0.16471	0.07830	−2.20	0.029	−0.3125	−0.1670
Serum FGF23	0.00353	0.00160	2.20	0.030	0.0003	0.0067
Hypertension	0.16586	0.07691	2.16	0.033	−0.0394	0.3178
Serum vitamin D3	−0.00432	0.00202	−2.15	0.034	−0.0083	−0.0003
Parathyroid hormone	0.00253	0.00152	1.66	0.099	−0.0005	0.0056
Diabetes mellitus	0.07369	0.08706	0.85	0.399	−0.0983	0.2456
Body mass index	0.00623	0.00951	0.65	0.514	−0.0126	0.0250

Abbreviations are the same as shown in Table 1.

There were significant relationships between HFpEF and PWW, age, central systolic BP, eGFR, Agatston score, sex, serum FGF23, hypertension, and vitamin D3. PWV, age, central systolic BP, Agatston Score, FGF23 plasma level, and hypertension all revealed significant positive relations with HFpEF. Plasma levels of VD3 as well as of eGFR and

male sex had significant negative relations with HFpEF. The phosphate serum level had significant regressions with female sex and the plasma level of FGF23 (r = 0.234, $p = 0.016$ and r = 0.343, $p = 0.001$, respectively).

Multivariable logistic regression analysis was performed for the independent variable, HFpEF. There were significant collinearities as follows: between PWV and age (r = 0.937, $p < 0.001$); between PWV and eGFR (r = −0.453, $p < 0.001$); between PWV and FGF23 (r = 0.339, $p < 0.001$); between PWV and vitamin D3 (r = −0.219, $p = 0.024$); and between FGF23 and vitamin D3 (r = −0.468, $p < 0.001$). Higher PWV values, hypertension, and male sex were selected as dependent variables for collinearities.

The analysis revealed that higher PWV values and hypertension were significant predictors for HFpEF, but not male sex (Table 4).

Table 4. Multiple logistic regression analyses for HFpEF. n = 158, F = (3, 154) = 15.03, $p < 0.0001$, R-squared = 0.2265.

HFpEF	Coef.	Std. Err.	t	$p > \|t\|$	95% Conf. Interval	
Higher PWV value	0.39451	0.06696	5.89	<0.001	0.2622	0.5270
Hypertension	0.15155	0.06972	2.17	0.031	0.0132	0.2893
Sex (male)	−0.10425	0.06823	−1.53	0.129	−0.2390	0.0305
-cons	−0.08095	0.07505	1.08	0.283	−0.0674	0.2291

Abbreviations are the same as shown in Table 1.

4. Discussion

Heart failure with preserved ejection fraction (HFpEF) is becoming increasingly recognized as a major public health concern worldwide [1–4]. Some HFpEF patients present with obesity and/or diabetes mellitus (DM), which often results in atherosclerosis and LV hypertrophy [1,2,5,6]. Another phenotype of HFpEF is frequently seen in elderly patients with CKD, as Cohen et al. reported [1,2]. Elderly people with CKD frequently present with aortic calcification similar to hemodialysis patients [7–9]. We recently reported that a deterioration of phosphate homeostasis, observed by measuring the serum levels of FGF23 and VD3, leads to arterial calcification, and could produce arterial stiffness, which intensifies LV afterload [20]. We therefore investigated whether arterial stiffness due to phosphate retention could lead to HFpEF.

Table 1 reveals that the HFpEF group indicated the following characteristics: higher age; female sex; renal dysfunction; higher systolic BP; higher pulse pressure; inflammation; higher BNP levels; higher aortic calcification scores; higher PWV; higher central pulse pressure; and higher cardiac afterload markers, including augmentation pressure and central systolic BP. The HFpEF group further presented hypertension associated factors and diastolic dysfunction markers, as documented in the ESC guidelines: LV thickness; concentric hypertrophy; decreased e′; increased E/e′; LA larger dimension; and a history of atrial fibrillation [4]. The HFpEF group moreover had increasing plasma levels of FGF23 and decreasing plasma levels of VD3. We can therefore understand that the effects of phosphate retention on arterial stiffness [20] and the pathological mechanism of HFpEF are related.

Vlachopoulos et al. reported that PWV is a gold standard for measuring arterial stiffness, and that arterial stiffness is causative of pulsatile afterload [10,22]. Arterial wave reflections increase according to the degree of arterial stiffness, leading to the incrementation of mid-to-late systolic load and subsequent LV abnormalities, including LV concentric remodeling and myocardial hypertrophy [10,22].

Arterial stiffness was intensified according to age and renal dysfunction degree, as is shown in Figure 1. The plasma levels of FGF23 and VD3 dovetailed with age and renal dysfunction, as we and others have presented [13,16–20]. The loss of nephrons due to aging and CKD causes a phosphate excretion disorder in the renal proximal tubules [8,13–17].

Phosphate retention is an important cause of arterial calcification [13–17]. Once the concentration of calcium and phosphate ions exceeds the blood saturation level, because the

extracellular fluid is super-saturated in terms of phosphate and calcium ions, an increase in the phosphate concentration can trigger precipitation of calcium-phosphate [13,30,31]. Calcium-phosphate precipitated upon an increase in the blood phosphate concentration is then absorbed by serum protein fetuin-A to form colloidal nanoparticles called calciprotein particles (CPPs). CPPs in the blood can induce cell damage, ectopic calcification, and inflammatory responses [13,30,31].

Increased FGF23 increases phosphate excretion per nephron; it therefore compensates for any reductions in nephron number, and as a result, it maintains phosphate homeostasis [13,15]. Increased phosphaturia is independently associated with a decline in eGFR in stage 2–3 CKD patients with normal blood phosphate levels [13,15]. Phosphate retention, which is a trigger for arterial calcification, may be launched in the earliest stages of the aging process [12–15].

This study further presents that the more arterial stiffness there is, the more augmentation pressure increases and the more central systolic BP increases, both of which are markers of LV afterload (Figure 2). This intensifies LV pulsatile afterload, indicated by central systolic BP, and has a significant relationship with LV hypertrophy, which leads to LV diastolic dysfunction (e′ and E/e′), as we presented in our results section. Leite et al. reported that LV diastolic dysfunction is induced by an increased afterload in the healthy hearts of rabbits and dogs [32]. Roy et al. reported that plasma level of FGF23 was increased in patients with HFpEF, and it was associated with a low survival rate [33]. By indicating the relationship between PWV and HFpEF judgement parameters, our study suggests that arterial stiffness leads to LV concentric hypertrophy, and finally to HFpEF (Figures 3 and 4).

Table 2 shows that elderly females, rather than males, more commonly present with HFpEF [1,2,7]. Elderly females demonstrated significant characteristics of arterial stiffness shown by an increased percent of AI, compared with males, as Goto et al. [7] and we presented. Females showed significantly higher levels of serum phosphate and lower bone mineral density levels than those seen in males. Bone is a safe and important depository for phosphate [8,11–14]. Osteoporosis patients have disadvantages in the management of phosphate; therefore, elderly females may have more arterial stiffness and HFpEF than males.

The diabetes mellitus rate was not significantly different between the HFpEF and the non-HFpEF groups, although there were relatively few patients with DM and/or obesity. DM patients had higher Agatston and calcification volume scores than non-DM patients in this study. Cohen et al. showed that obese and diabetic patients had a higher pulsatile arterial load, increased concentric LV hypertrophy, and an increase in E/e′ [2,6]. Chirinos et al. reported that in HFpEF patients with DM, there was an increase in arterial stiffness, PWV, and LV mass [6]. Patients with DM or obesity, as well as elderly people with CKD, all have increased PWV values [6,7,10]. It is natural to assume, therefore, that arterial stiffness should be considered an important mechanism of HFpEF [6–10,20]

Single linear regression analyses showed that PWV, age, central systolic BP, eGFR, Agatston calcification scores, female sex, serum FGF23 levels, hypertension, and serum VD3 levels all revealed significant relationships with the independent factor, HFpEF. All of these factors are related to phosphate retention or arterial stiffness. There were many significant collinearities in the phosphate retention-related factors. This means that phosphate retention is strongly related to HFpEF. We determined that PWV, hypertension, and sex difference were independent factors for HFpEF by considering their collinearity. Multivariable logistic regression analyses revealed that both higher PWV values and hypertension were important predictors for HFpEF (Table 4). This study reveals that the main triggering mechanism of HFpEF is arterial stiffness, effected by phosphate retention.

The mechanism and/or causation of HFpEF is clearly different from that of heart failure with reduced ejection fraction (HFrEF), which is a result of myocardial dysfunction. This study presents that phosphate retention, due to a loss of nephrons associated with

aging and CKD, accelerates the aging process. Increasing arterial stiffness leads to LV diastolic dysfunction and/or HFpEF by amplifying LV pulsatile afterload.

It is important to recognize phosphate retention using FGF23 and VD3 measurements in the early stages of CKD. An increase in the former and a decrease in the latter indicate the actual degree of phosphate retention. We propose that Klotho gene up-regulation therapy, phosphate regulating medications, phosphate restriction diets, and osteoporosis therapies may all be effective for phosphate control, especially in females [34,35]. Preventing phosphate retention should be a new clinical target for treating aging and aging related diseases [13,16,34,35].

5. Limitations

This is a cross-sectional observation study. There is a limited discussion of causes and effects. Our patients were mainly elderly patients suspected of heart failure. There were no patients who needed invasive hemodynamic measurements during exercise. There was only a limited discussion about younger patients and obese or diabetic patients. We used BaPWV tests to estimate central blood pressure, especially LV afterload related markers; however, cardio-ankle vascular index has been used by some as a marker for arterial stiffness.

6. Conclusions

The degree of arterial stiffness increases due to aging and CKD; furthermore, arterial stiffness is an important predictor for HFpEF. Arterial stiffness produces LV pulsatile afterload, which leads to LV concentric hypertrophy and LV diastolic dysfunction. Higher PWV values, as well as hypertension, are important predictors for HFpEF. Arterial stiffness, due to phosphate retention, should be a new therapeutic target for treating aging-related diseases, including HFpEF, as well as preventing cardiac mortality and morbidity overall.

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Informed Consent Statement: The significance and content of this study were explained to all patients, and they signed a consent form.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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