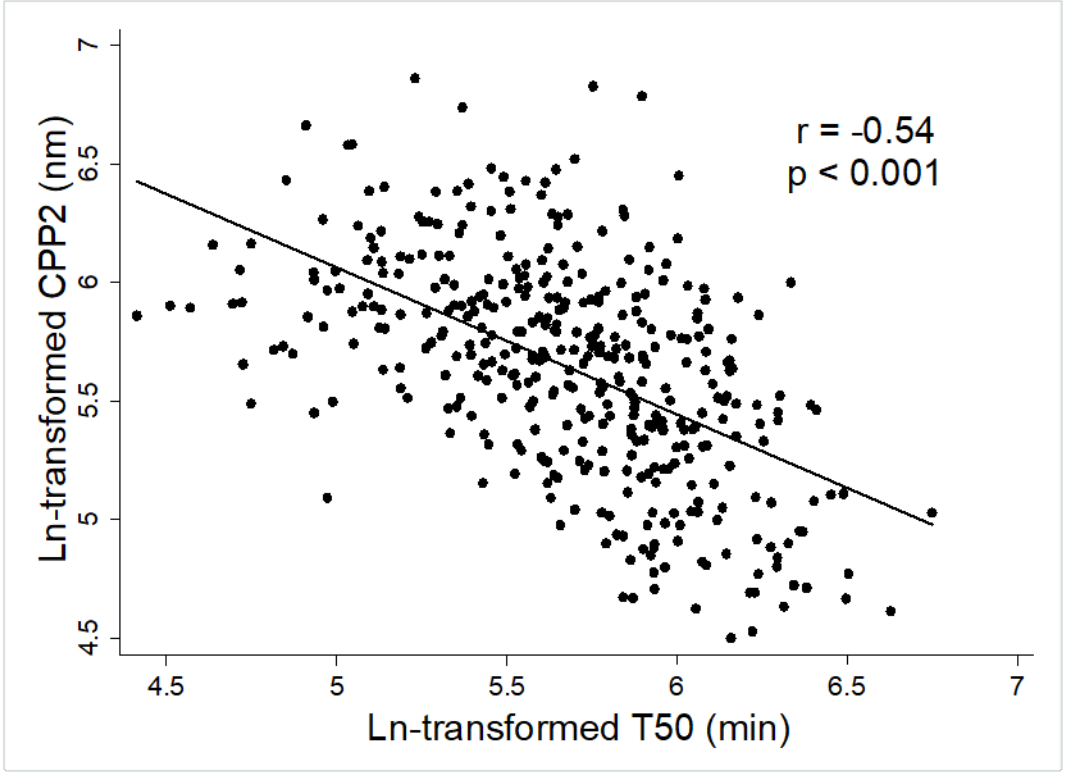


Figure S1. Correlation between CPP2 size and T_{50}. Pearson correlation was used after the logarithmic transformation of CPP2 size and T_{50}.

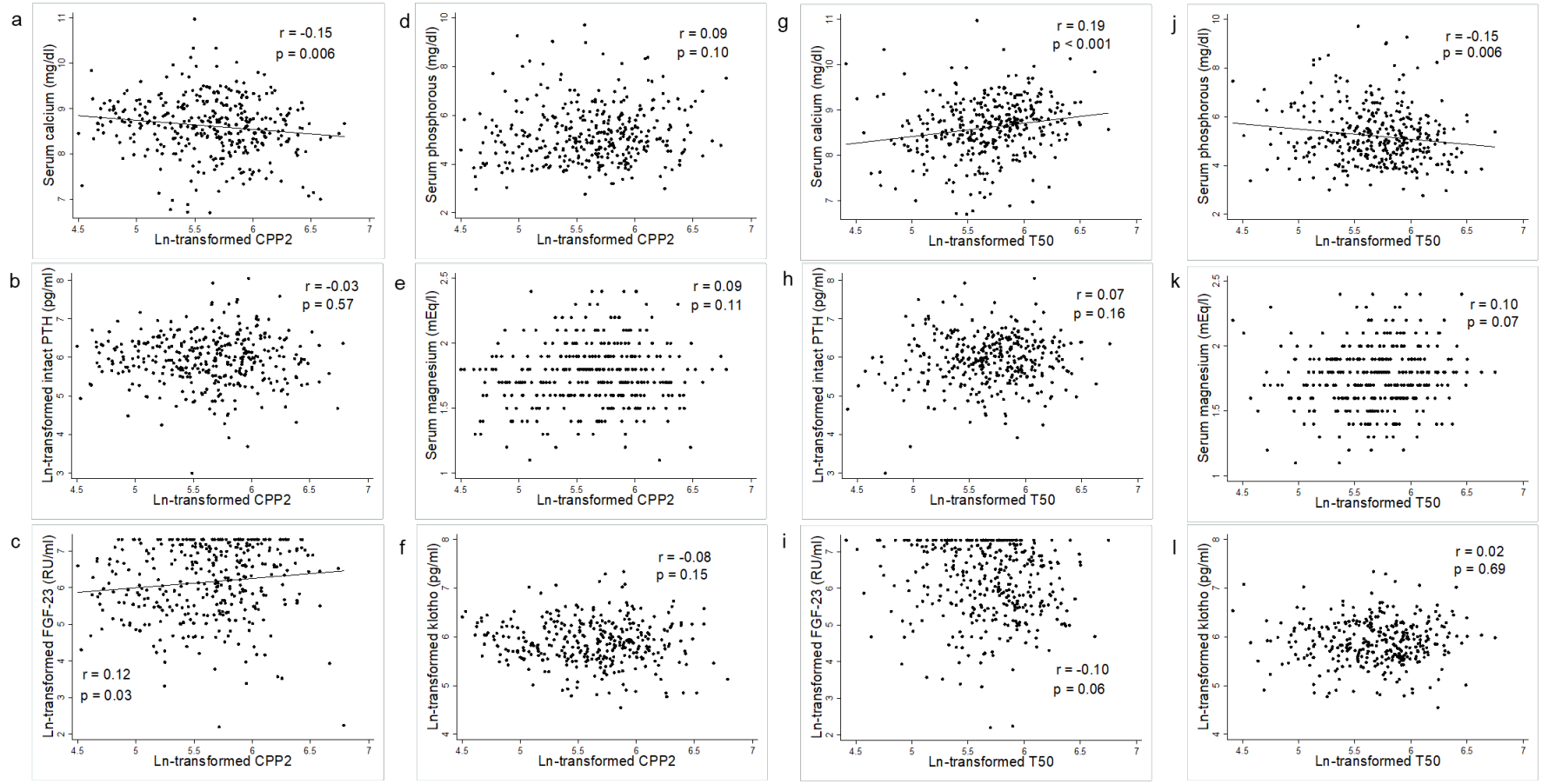


Figure S2. Scatterplots of CPP2 size and T_{50} with serum markers of mineral metabolism. (a-f) CPP2 size: larger CPP2 was correlated with lower serum calcium (a) and higher FGF23 levels (c); CPP2 size was not correlated with intact PTH (b), serum phosphorous (d), magnesium (e) or soluble klotho (f). (g-l) T_{50}: higher T_{50} was correlated with higher serum calcium (g) and lower phosphorous levels (j); T_{50} was not correlated with intact PTH (h), FGF-23 (i), serum magnesium (k) or soluble klotho (l). Pearson correlation was used after the logarithmic transformation of CPP2 size, T_{50}, intact PTH, FGF-23, and soluble klotho. Abbreviations: CPP2, secondary calciprotein particle; T_{50}, half maximal transformation of primary to secondary calciprotein particle; PTH, parathyroid hormone; FGF-23, fibroblast growth factor-23; klotho, soluble klotho.

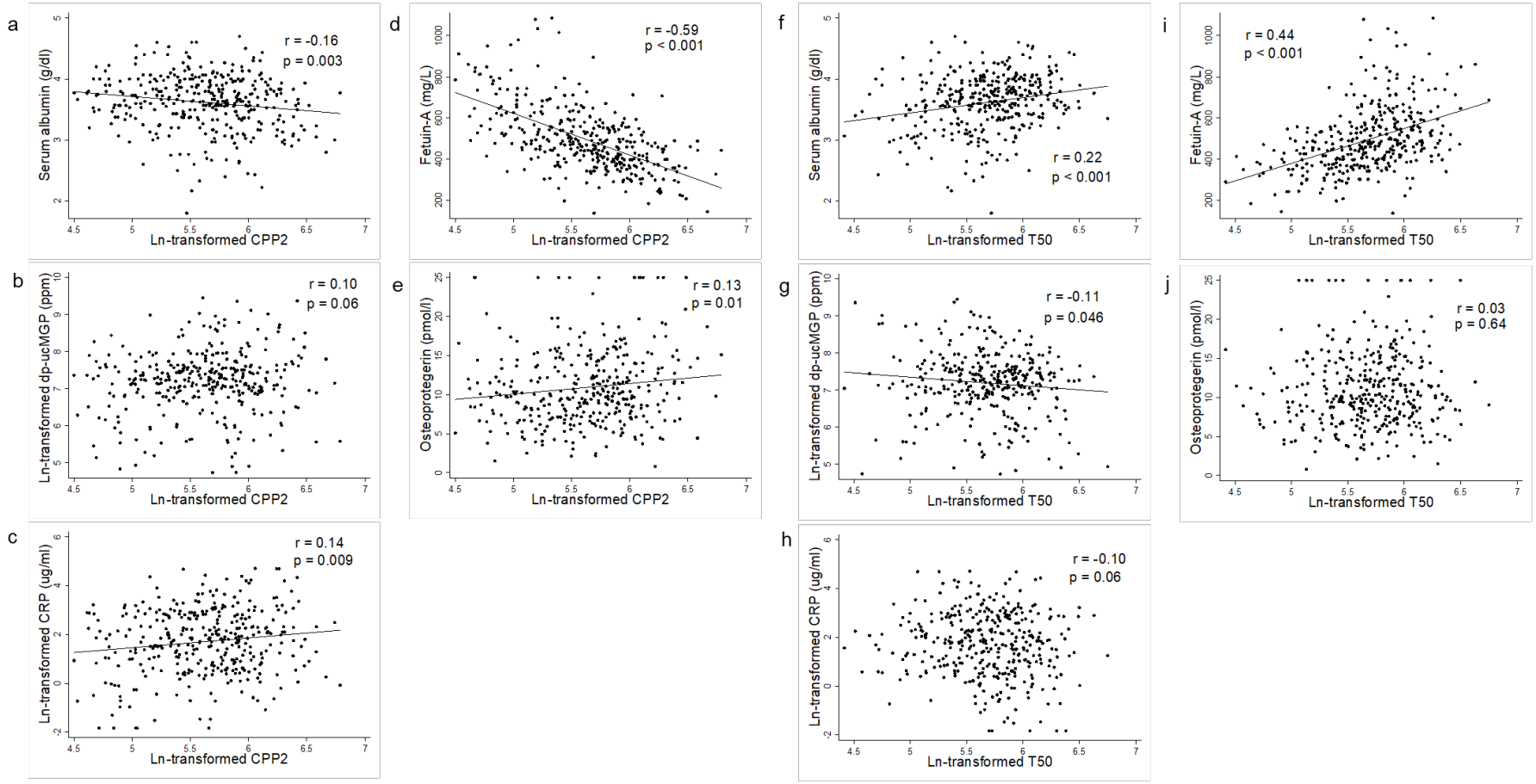
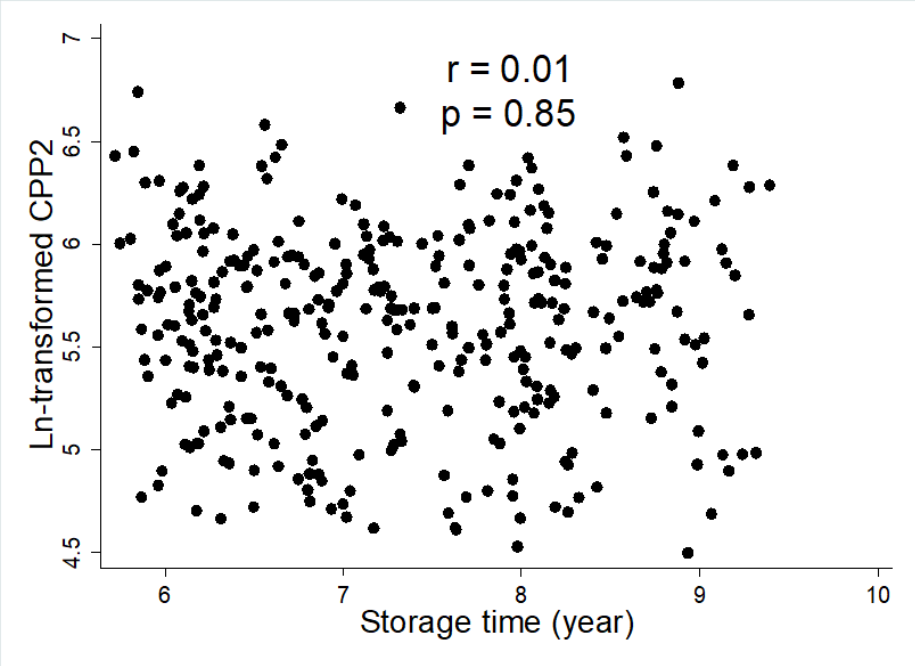


Figure S3. Scatterplots of CPP2 size and T_{50} with circulating inhibitors of arterial calcification and C-reactive protein. (a-e) CPP2 size: larger CPP2 was correlated with lower serum albumin (a), higher CRP (c), lower fetuin-A (d), and higher osteoprotegerin levels (e); CPP2 size was not correlated with dp-ucMGP (b). (f-j) T_{50}: higher T_{50} was correlated with higher serum albumin (f), lower dp-ucMGP (g), and higher fetuin-A (i). T_{50} was not correlated with CRP (h) or osteoprotegerin (j). Pearson correlation was used after the logarithmic transformation of CPP2 size, T_{50}, dp-ucMGP, and C-reactive protein. Abbreviations: CPP2, secondary calciprotein particle; T_{50}, half maximal transformation of primary to secondary calciprotein particle; dp-ucMGP, Dephosphorylated and uncarboxylated matrix Gla protein; FGF23, fibroblast growth factor-23; CRP, C-reactive protein.



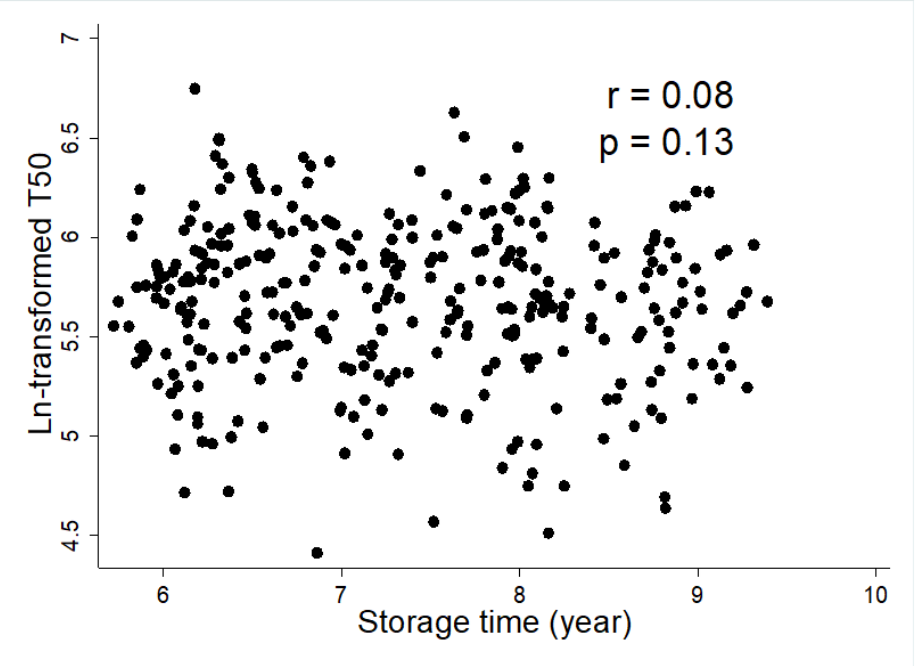


Figure S4. Correlation of CPP2 size and T_{50} with storage duration. Pearson correlation was used after the logarithmic transformation of CPP2 size and T_{50}.

Item S1. Supplementary Methods

Detailed description on measurement of calciprotein particle transformation

At the baseline visit, serum was collected on a non-HD day after ~8 hours of fasting and stored at -80°C. CPP transformation of serum was measured using dynamic light scattering, which determines the size distribution of nanoparticles in solution. The details of the assay are previously described.[1] After adding concentrated calcium and phosphate solutions (10 mM calcium and 6 mM phosphate) in the serum, we measured hydrodynamic radius of CPPs in a 384-well microplate at a constant temperature of 37°C for 10 hours using DynaPro Plate Reader II (Wyatt Technology, Santa Barbara, CA, USA), which is an automatic, temperature controlled microplate reader using dynamic light scattering. Using the assay, we previously found that larger CPP2 aggregates was significantly associated with arterial calcification in patients with CKD stage 4-5 after adjusting for age, diabetes mellitus, serum calcium and phosphate.[1] In current study, serum samples were measured in duplicate, in multiple microplates. Of the 411 samples measured, transformation from CPP1 to CPP2 was not observed in 9 samples after 10 hours, so neither CPP2 size or T_{50} was obtained. Equipment error occurred in 14 samples resulting missing data points after ~6 hours. Equipment was later resumed and CPP2 size was obtained, but T_{50} was not (Figure 1). As a result, CPP2 size was measured in 402 samples, and T_{50} in 388. For quality control, we used a size standard (hydrodynamic radius=500 nm, Polysciences Inc, Warrington, PA, USA) and control human serum (i.e. serum collected from the same individual at one setting). The coefficient of variation (CV) was 3% for a size standard. Using the control human serum, the CV for the size of CPP2 aggregates was 13% and for T_{50} was 10%. Median storage duration of the serum was 7.2 years [interquartile range (IQR) 6.5-8.1]. Using Pearson correlation, there was no statistical evidence that storage duration was associated with either CPP2 ($p=0.85$) or T_{50} ($p=0.13$; Figure S4).

Detailed description on measurement of outcome variables

Outcome variables were coronary arterial calcification (CAC) score, thoracic aortic calcification (TAC) score, pulse wave velocity (PWV), ankle brachial index (ABI), and all-cause mortality. Arterial calcification was measured in coronary arteries and the thoracic aorta using computed tomography (CT; Toshiba Aquilon One, Japan) at the baseline visit.[2] CAC score was quantified based on CT using Agatston score.[3] TAC score was calculated as the sum of calcium scores from the ascending and descending thoracic aorta. Of the 402 participants with available data on CPP2, 291 participants had CT examination and available CAC scores (Figure 1). TAC scores were available in 203 participants. Arterial stiffness was defined as high PWV or high ABI. PWV measurements were taken in the carotid and femoral arteries, with the participant in the supine position, using Sphygmocor PVx system (Actor Medical, Australia). As previously described,[3] PWV measurement was performed by trained research staff. Quality control was assessed using quality indices and operator index. Additional internal quality control included review of study procedures by operator every 6 months to ensure adherence to the protocol. PWV was measured in 311 participants at baseline and also in 154 participants at year 1. ABI was calculated as the ratio of ankle to brachial systolic blood pressure, and the lower of the bilateral ABI measurements was used for analyses.[4] Participants with an ABI≤0.9 (n=23) were excluded from the analyses involving ABI because an ABI≤0.9 indicates the presence of peripheral arterial

disease.[5] High ABI was defined as an ABI>1.4 or having incompressible vessels, and normal ABI as >0.9 and ≤1.4.[6] Mortality data were ascertained from the United States Renal Data System.

Detailed description on the measurement of covariates

Serum markers of mineral metabolism included serum calcium, phosphate, intact parathyroid hormone (PTH), fibroblast growth factor-23 (FGF23), and soluble klotho. Circulating inhibitors of arterial calcification included serum albumin,[7,8] dephosphorylated and uncarboxylated matrix glutamate (Gla) protein (dp-ucMGP),[9,10] osteoprotegerin,[11] and fetuin-A[12]. Serum calcium, phosphate, intact PTH, albumin, and hemoglobin levels were averaged using 3-months of lab values collected before a routine dialysis session. Using fasting serum samples obtained at baseline on a non-HD day and enzyme-linked immunosorbent assays, we measured high sensitivity C-reactive protein (CV=7%; BNII Nephelometer; Siemens Healthcare, Germany), dp-ucMGP (CV=11%; VitaK BV, Maastricht, Netherlands), osteoprotegerin (CV=9%; Alpaco Diagnostics, Salem, NH, USA), fetuin-A (CV=18%; Epitope Diagnostics, San Diego, CA, USA), C-terminal FGF23 (CV=13%; upper threshold=1500 RU/ml; Immutopics, San Clemente, CA, USA), and soluble klotho (CV=2.3%; Immuno-biological Laboratories, Takasaki, Japan).

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Table S1. Baseline participant characteristics by CAC status among incident HD participants from PACE cohort

	CAC=0 (n=105)	CAC>0 (n=178)
Age, year	46 ± 13	59 ± 12
Women, n (%)	45 (43%)	67 (37%)
African American, n (%)	96 (91%)	115 (65%)
Body Mass Index, kg/m^2	30 ± 7.4	28 ± 6.9
History of smoking, n (%)	58 (55%)	113 (64%)
Education, less than grade 11, n (%)	48 (46%)	62 (35%)
Diabetes mellitus, n (%)	57 (54%)	101 (57%)
Coronary artery disease, n (%)	23 (22%)	71 (40%)
Serum calcium, mg/dL	8.6 ± 0.7	8.6 ± 0.7
Serum phosphorous, mg/dL	5.3 ± 1.2	5.2 ± 1.1
Serum magnesium, mEq/L	1.8 ± 0.2	1.8 ± 0.2
Intact parathyroid hormone, pg/mL	442 (332-606)	332 (242-577)
Serum albumin, g/dL	3.6 ± 0.5	3.6 ± 0.4
Hemoglobin, g/dL	10.7 ± 1.3	10.7 ± 1.2
LDL, mg/dL	86 (62-104)	81 (60-112)
Fetuin-A, mg/L	525 ± 167	486 ± 153
Dp-ucMGP, ppm	1265 (762-1905)	1459 (992-2067)
Osteoprotegerin, pmol/L	9.4 ± 4.1	12.0 ± 4.9
FGF23, RU/ml	548 (197-1111)	630 (277-1243)
Klotho, pg/mL	381 (273-511)	362 (281-493)
C-reactive protein, μg/mL	4.7 (1.8-10.4)	5.9 (2.6-17)
CPP1, nm	55 ± 8	55 ± 9
CPP2, nm	292 (203-389)	288 (201-371)
T_{50}, min	304 (246-377)	316 (228-406)
Calcium-based phosphate binder, n (%)	39 (37%)	64 (36%)
Vitamin D therapy, n (%)	83 (79%)	134 (75%)
RAAS blockade, n (%)	45 (51%)	69 (42%)

Abbreviations: LDL, low density lipoprotein; RAAS, renin-angiotensin-aldosterone system; dp-ucMGP, Dephosphorylated and uncarboxylated matrix Gla protein; FGF23, fibroblast growth factor-23; CPP1, primary calciprotein particle; CPP2, secondary calciprotein particle; T_{50}, half-maximal time of transformation from CPP1 to CPP2. Note: If normally distributed, values for continuous variables with normal distribution are provided as mean ± standard deviation. Otherwise, they are provided as median (interquartile range). Categorical variables are presented as absolute number with percentage.

Table S2. Longitudinal association of CPP2 size and T_{50} with repeated measures of pulse wave velocity (1 year follow up)

	CPP2 (per 100 nm)			T_{50} (per 100 min)		
	n	% Change (95% CI)	p	n	% Change (95% CI)	p
Unadjusted	311	2.06 (-0.42, 4.60)	0.11	298	0.83 (-1.74, 3.46)	0.53
Model 1	306	0.19 (-1.84, 2.25)	0.86	293	0.50 (-1.63, 2.67)	0.65
Model 2	302	-0.19 (-2.29, 1.94)	0.86	289	0.95 (-1.22, 3.17)	0.40
Model 3	302	-0.23 (-2.64, 2.23)	0.85	289	1.03 (-1.41, 3.53)	0.10
Model 4	289	-0.10 (-2.20, 2.05)	0.93	276	1.16 (-1.08, 3.45)	0.31
Model 5	305	0.27 (-1.79, 2.37)	0.80	292	0.43 (-1.72, 2.62)	0.70

Model 1: adjusted for demographics (age, sex, race), comorbidities (diabetes, coronary artery disease)
Model 2: adjusted for demographics, comorbidities, serum markers of mineral metabolism (calcium, phosphorous, FGF-23)
Model 3: adjusted for demographics, comorbidities, serum albumin, fetuin-A
Model 4: adjusted for demographics, comorbidities, dp-MGP, osteoprotegerin
Model 5: adjusted for demographics, comorbidities, C-reactive protein

Table S3. Associations of CPP2 size and T50 with pulse wave velocity at baseline and after 1 year follow up, presented in beta coefficients

	CPP2 size (per 100 nm)			T_{50} (per 100 min)		
PWV (m/s) at baseline						
	n	β (95% CI)	p	n	β (95% CI)	p
Unadjusted	311	0.02 (-0.01, 0.04)	0.21	298	0.02 (-0.01, 0.04)	0.26
Model 1	306	-0.0002 (-0.02, 0.02)	0.99	293	0.01 (-0.01, 0.03)	0.38
Model 2	302	-0.003 (-0.03, 0.02)	0.78	289	0.01 (-0.01, 0.04)	0.25
Model 3	302	-0.01 (-0.03, 0.02)	0.65	289	0.02 (-0.01, 0.04)	0.19
Model 4	289	-0.003 (-0.03, 0.02)	0.78	276	0.02 (-0.01, 0.04)	0.12
Model 5	305	-0.002 (-0.02, 0.02)	0.88	292	0.01 (-0.01, 0.04)	0.33
Repeated measures of pulse wave velocity (1 year follow up, m/s)						
	n	β (95% CI)	p	n	β (95% CI)	p
Unadjusted	311	0.02 (-0.004, 0.04)	0.11	298	0.01 (-0.02, 0.03)	0.53
Model 1	306	0.002 (-0.02, 0.02)	0.86	293	0.005 (-0.02, 0.03)	0.65
Model 2	302	-0.002 (-0.02, 0.02)	0.86	289	0.01 (-0.01, 0.03)	0.40
Model 3	302	-0.002 (-0.03, 0.02)	0.85	289	0.01 (-0.01, 0.03)	0.10
Model 4	289	-0.001 (-0.02, 0.02)	0.93	276	0.01 (-0.01, 0.03)	0.31
Model 5	305	0.003 (-0.02, 0.02)	0.80	292	0.004 (-0.02, 0.03)	0.70

Model 1: adjusted for demographics (age, sex, race), comorbidities (diabetes, coronary artery disease)
Model 2: adjusted for demographics, comorbidities, serum markers of mineral metabolism (calcium, phosphorous, FGF-23)
Model 3: adjusted for demographics, comorbidities, serum albumin, fetuin-A
Model 4: adjusted for demographics, comorbidities, dp-MGP, osteoprotegerin
Model 5: adjusted for demographics, comorbidities, C-reactive protein

Table S4. Cross-sectional association of CPP2 size and T_{50} with arterial stiffness measured by high ABI (high ABI vs. normal ABI)

	CPP2 (per 100 nm)			T_{50} (per 100 min)		
	n	PR (95% CI)	p	n	PR (95% CI)	p
Unadjusted	236	1.10 (0.86, 1.39)	0.45	226	0.85 (0.68, 1.06)	0.15
Model 1	236	1.09 (0.88, 1.35)	0.42	226	0.84 (0.66, 1.06)	0.14
Model 2	234	1.09 (0.86, 1.37)	0.47	224	0.86 (0.67, 1.08)	0.17
Model 3	234	1.06 (0.80, 1.42)	0.67	224	0.88 (0.63, 1.21)	0.43
Model 4	225	1.08 (0.87, 1.34)	0.47	215	0.82 (0.64, 1.05)	0.12
Model 5	234	1.09 (0.87, 1.36)	0.45	224	0.84 (0.66, 1.06)	0.14

Abbreviations: ABI, ankle brachial index; PR, prevalence ratio

Model 1: adjusted for demographics (age, sex, race), comorbidities (diabetes, coronary artery disease)

Model 2: adjusted for demographics, comorbidities, serum markers of mineral metabolism (calcium, phosphorous, FGF-23)

Model 3: adjusted for demographics, comorbidities, serum albumin, fetuin-A

Model 4: adjusted for demographics, comorbidities, dp-MGP, osteoprotegerin

Model 5: adjusted for demographics, comorbidities, C-reactive protein

Table S5. Cross-sectional association of CPP2 size and T_{50} with arterial calcification (CAC and TAC) and arterial stiffness (PWV), stratified by the status of diabetes mellitus and coronary artery disease

	CPP2 (per 100 nm)		T_{50} (per 100 min)	
CAC score				
	Coefficient (95% CI)	n	Coefficient (95% CI)	n
DM	-0.01 (-0.44, 0.41)	161	0.14 (-0.38, 0.67)	158
No DM	-0.17 (-0.54, 0.20)	130	-0.21 (-0.64, 0.22)	125
CAD	0.23 (-0.21, 0.66)	97	-0.28 (-0.81, 0.25)	94
No CAD	-0.22 (-0.59, 0.15)	194	0.05 (-0.38, 0.49)	189
TAC score				
	Coefficient (95% CI)	n	Coefficient (95% CI)	n
DM	-0.08 (-0.68, 0.53)	114	0.26 (-0.49, 1.00)	113
No DM	0.25 (-0.49, 0.98)	89	-0.80 (-1.95, 0.35)	84
CAD	0.41 (-0.26, 1.08)	70	-0.71 (-1.58, 0.17)	68
No CAD	-0.13 (-0.70, 0.45)	133	0.40 (-0.56, 1.36)	129
PWV (m/s)				
	% Change (95% CI)	n	% Change (95% CI)	n
DM	-0.62 (-3.73, 2.59)	162	2.22 (-1.39, 5.95)	159
No DM	0.37 (-2.57, 3.39)	144	-0.06 (-2.99, 2.95)	134
CAD	-0.09 (-3.80, 3.77)	106	1.49 (-2.41, 5.55)	102
No CAD	1.14 (-2.02, 4.39)	200	0.47 (-2.94, 4.00)	191

Adjusted for demographics (age, sex, race)

Table S6. Cross sectional association of CPP2 size and T_{50} categories with arterial calcification (CAC and TAC) and arterial stiffness (PWV) (Sensitivity analyses)

	Small CPP2 & high T_{50} (n=135)		Small CPP2 & low T_{50} (n=53)		Large CPP2 & high T_{50} (n=58)		Large CPP2 & low T_{50} (n=141)	
CAC score								
	Coefficient	p	Coefficient (95% CI)	p	Coefficient (95% CI)	p	Coefficient (95% CI)	p
Unadjusted (n=283)	Reference		0.66 (-1.04, 2.36)	0.44	0.75 (-0.93, 2.42)	0.38	-0.17 (-1.49, 1.15)	0.80
Model 1 (n=283)	Reference		1.34 (-0.02, 2.69)	0.05	0.14 (-1.14, 1.42)	0.83	-0.27 (-1.36, 0.81)	0.62
Model 2 (n=278)	Reference		1.19 (-0.21, 2.58)	0.10	-0.09 (-1.37, 1.19)	0.89	-0.56 (-1.74, 0.62)	0.35
Model 3 (n=278)	Reference		1.08 (-0.28, 2.44)	0.12	0.01 (-1.30, 1.31)	0.99	-0.75 (-2.03, 0.53)	0.25
Model 4 (n=264)	Reference		1.58 (0.13, 3.03)	0.03	-0.10 (-1.39, 1.19)	0.88	-0.35 (-1.51, 0.81)	0.55
Model 5 (n=281)	Reference		1.31 (-0.001, 2.62)	0.05	0.07 (-1.25, 1.39)	0.92	-0.38 (-1.53, 0.76)	0.52
TAC score								
	Coefficient	p	Coefficient (95% CI)	p	Coefficient (95% CI)	p	Coefficient (95% CI)	p
Unadjusted (n=197)	Reference		-0.96 (-3.99, 2.06)	0.53	2.16 (-0.58, 4.91)	0.12	0.55 (-1.45, 2.55)	0.59
Model 1 (n=197)	Reference		0.02 (-2.31, 2.36)	0.99	0.75 (-1.25, 2.74)	0.46	0.31 (-1.27, 1.89)	0.70
Model 2 (n=195)	Reference		-0.26 (-2.63, 2.11)	0.83	0.39 (-1.69, 2.46)	0.71	-0.13 (-1.81, 1.55)	0.88
Model 3 (n=195)	Reference		-0.33 (-2.74, 2.08)	0.79	0.68 (-1.59, 2.94)	0.56	-0.19 (-2.38, 2.00)	0.86
Model 4 (n=182)	Reference		-0.17 (-2.60, 2.25)	0.89	0.36 (-1.73, 2.45)	0.74	-0.18 (-1.78, 1.43)	0.83
Model 5 (n=196)	Reference		0.002 (-2.46, 2.46)	>0.99	0.72 (-1.38, 2.81)	0.50	0.26 (-1.43, 1.94)	0.77
Pulse wave velocity								
	% Difference	p	% Difference (95% CI)	p	% Difference (95% CI)	p	% Difference (95% CI)	p
Unadjusted	Reference		-4.61 (-14.35, 6.24)	0.39	8.37 (-2.36, 20.27)	0.13	-0.75 (-8.42, 7.56)	0.85

(n=297)							
Model 1 (n=292)	Reference	0.96 (-8.17, 11.01)	0.84	2.04 (-7.01. 11.98)	0.67	-1.54 (-8.11. 5.50)	0.66
Model 2 (n=288)	Reference	0.20 (-9.05, 10.40)	0.97	2.06 (-7.12, 12.15)	0.67	-2.41 (-9.19, 4.87)	0.50
Model 3 (n=288)	Reference	-0.51 (-9.81, 9.75)	0.92	0.51 (-8.86. 10.86)	0.92	-4.22 (-11.70, 3.90)	0.30
Model 4 (n=275)	Reference	-0.09 (-9.53, 10.74)	0.99	4.14 (-5.35, 14.58)	0.40	-2.26 (-9.04, 5.02)	0.53
Model 5 (n=291)	Reference	0.84 (-8.32, 10.92)	0.86	1.99 (-7.09, 11.96)	0.68	-1.92 (-8.57, 5.21)	0.59

For CAC and TAC scores, we transformed calcification score [Ln(calcification score+1)], then used Tobit regression with left censoring at 0 and bootstrap techniques with 999 repetitions.

Model 1: adjusted for demographics (age, sex, race), comorbidities (diabetes, coronary artery disease)
Model 2: adjusted for demographics, comorbidities, serum markers of mineral metabolism (calcium, phosphorous, FGF-23)
Model 3: adjusted for demographics, comorbidities, serum albumin, fetuin-A
Model 4: adjusted for demographics, comorbidities, dp-MGP, osteoprotegerin
Model 5: adjusted for demographics, comorbidities, C-reactive protein