European Heart Journal (2012) **33**, 2873–2881
doi:10.1093/eurheartj/ehs152

Impact of serum calcium and phosphate on coronary atherosclerosis detected by cardiac computed tomography

Sanghoon Shin[1†], Kwang-Joon Kim[2,3†], Hyuk-Jae Chang[1,4*], Iksung Cho[1], Young Jin Kim[5], Byoung-Wook Choi[5], Yumie Rhee[2], Sung-Kil Lim[2], Woo-In Yang[1], Chi-Young Shim[1], Jong-Won Ha[1], Yangsoo Jang[1], and Namsik Chung[1]

[1]Division of Cardiology, Severance Cardiovascular Hospital, Yonsei University College of Medicine, Yonsei University Health System, Seoul, South Korea; [2]Division of Endocrinology, Department of Internal Medicine, Yonsei University College of Medicine, Yonsei University Health System, Seoul, South Korea; [3]Severance Executive Healthcare Clinic, Yonsei University Health System, Seoul, South Korea; [4]Severance Biomedical Science Institute, Yonsei University Health System, Seoul, South Korea; and [5]Division of Radiology, Severance Cardiovascular Hospital, Yonsei University College of Medicine, Yonsei University Health System, Seoul, South Korea

Received 3 November 2011; revised 18 April 2012; accepted 7 May 2012; online publish-ahead-of-print 19 June 2012

Aims High calcium (Ca), phosphate (P), and Ca–P product (CPP) are associated with cardiovascular disease in patients with chronic kidney disease. Whether this relationship persists in individuals with normal kidney function is not yet elucidated. We explored the relationship of serum Ca, P, and CPP to coronary atherosclerosis assessed by cardiac computed tomography angiography (cCTA) in participants with normal kidney function.

Methods and results This study included 7553 participants (52 ± 10 years, male 57%) with near-normal kidney function (estimated glomerular filtration rate > 60 mL/min/1.73 m^2) who underwent cCTA. The relationship of Ca, P, and CPP to coronary atherosclerosis [coronary artery Ca score (CACS) >100 and the presence of coronary artery disease (CAD)] was evaluated. Higher Ca, P, and CPP were significantly associated with CACS > 100 continuously [adjusted odds ratio (OR) per mg/dL: Ca 1.21, $P = 0.026$; P 1.29, $P < 0.001$; CPP 1.03, $P < 0.001$]. However, they correlate only weakly with the presence of CAD (OR: Ca 1.17, $P = 0.001$; P 1.05, $P = 0.173$; CPP 1.01, $P = 0.034$). This discrepancy was because calcified or mixed plaque and non-calcified plaque (NCP) were included in CAD. A significant relationship was demonstrated between calcified or mixed plaque and Ca, P, and CPP (OR: Ca 1.20, $P = 0.001$; P 1.13, $P = 0.003$; CPP 1.02, $P = 0.001$), but not NCP.

Conclusion Elevated serum levels of Ca, P, and CPP are significantly associated with the presence of calcified coronary atherosclerotic plaque. It is unclear if there is a causal relationship. This relationship is thought to contribute to vascular calcification, but is less closely associated with NCP.

Keywords Atherosclerosis • Computed tomography • Calcium • Phosphate

Introduction

Serum calcium (Ca) and inorganic phosphorous (P) levels are tightly regulated within a narrow range in healthy individuals, and maintenance of normal serum Ca and P levels is a pre-requisite for various physiological processes, including bone formation, vascular function, several metabolic pathways, and intracellular signalling. Therefore, abnormalities in Ca and P homeostasis have been directly or indirectly linked to various skeletal, endocrine, and cardiovascular disorders.[1–3] In clinical settings, abnormalities in Ca and P metabolism are most commonly found in individuals with impaired renal function. Several previous studies have shown that higher serum Ca and P levels are associated with unaccountably high rates of cardiovascular disease (CVD) in patients

† These authors contributed equally to this work as first authors.
* Corresponding author. Tel: +82 2 2228 8454; Fax: +82 2 393 2041, Email: hjchang@yuhs.ac

with chronic kidney disease (CKD),[4–6] and recent studies have suggested that Ca–P homeostasis plays a role in the pathogenesis of atherosclerosis, especially in the coronary arteries.[7,8]

Major consequences of elevated serum Ca and P in patients with CKD are vascular calcification and increased risk of cardiovascular morbidity and mortality.[9] In addition some previous studies suggested that even with normal kidney function, higher serum Ca is associated with increased cardiovascular morbidity. Large-scale epidemiological studies have shown that in middle-aged men, serum Ca levels are an independent, prospective risk factor for CVD,[10,11] and high-normal serum Ca levels are associated with increased cardiovascular mortality.[12] Similarly, recent studies also suggest that higher serum P is associated with abnormal vascular phenotypes, such as increased carotid intima-media thickness,[13] arterial stiffness,[14] and cardiovascular mortality.[15] Reports that Ca supplementation increases cardiovascular risk, especially the risk of myocardial infarction[16] have raised interest in the association between Ca, P, and CVD.

Coronary artery Ca score (CACS) is a good marker of atherosclerosis which represents the degree of atheromatous plaque burden,[17,18] and coronary computed tomography angiography (cCTA) has achieved high diagnostic accuracy for detecting coronary artery disease (CAD).[19–21] Therefore, we investigated the relationship of serum Ca and P with coronary atherosclerosis using non-invasive cCTA, a useful method in predicting cardiovascular disease[22,23] in participants with near-normal kidney function. Furthermore, we have also observed how the relationship between the two factors changes under the presence of various risk factors after considering the increase in the incidence of cardiovascular disease due to the risk factors such as diabetes.[24]

Methods

Study design and population

This observational retrospective single-centre study consisted of 8648 consecutive participants who underwent cCTA evaluation using 64-slice multi-detector computed tomography (MDCT) from January 2004 to April 2009 at Severance Hospital. Patients were referred for a variety of indications, including asymptomatic subject for a general health evaluation, evaluation of symptoms, signs of cardiac disease such as abnormal resting or stress electrocardiographic test, or asymptomatic patients with peripheral arterial disease, cerebrovascular disease, or multiple CAD risk factors. Symptoms included typical angina, atypical angina, dyspnea, and excessive fatigue. Participants were excluded if any one of the following criteria was met: (i) age $<$ 30 years ($n = 69$), (ii) modification of diet in renal disease (MDRD) glomerular filtration rate (GFR) under 60 mL/min/1.73 m^2 ($n = 958$), or (iii) insufficient medical records ($n = 68$). As a result, 7553 participants were finally included.

Multi-detector computed tomography protocol

Patients with initial heart rate higher than 65 beats/min before MDCT examination received a single oral dose of 50 mg metoprolol tartrate (Betaloc, Yuhan, Seoul, Korea) 1–2 h before CT examination unless β-adrenergic-blocking agents were contraindicated (overt heart failure or atrioventricular conduction abnormalities). Patients were scanned using a 64-slice CT scanner (Sensation 64; Siemens Medical Systems, Forchheim, Germany). Before the helical scan for CCTA, a non-enhanced prospective electrocardiogram (ECG)-gated scan was performed to measure CACS with the following parameters: rotation time of 330 ms, slice collimation of 0.6 mm, slice width of 3.0 mm, tube voltage of 120 kV, tube current of 50 mA, and table feed/scan of 18 mm. Afterward, cCTA was performed using retrospective ECG-gating with the following scan parameters: rotation time of 330 ms, slice collimation of 64 × 0.6 mm, tube voltage of 100–120 kV, tube current of 600–800 mA depending on patient size, table feed/scan of 3.8 mm, and pitch factor of 0.2. ECG-based tube current modulation was applied to 65% of the R–R interval. A real-time bolus-tracking technique was used to trigger the initiation of the scan. The total estimated average radiation dose for the multislice CT protocol (CACS + cCTA) was 10.9 ± 1.9 mSv. The average radiation doses were 1.7 ± 0.3 mSv for the CACS protocol and 8.8 ± 1.6 mSv for the CCTA protocol.

Contrast enhancement was achieved with 60 mL iopamidol (370 mg iodine/mL, Iopamiro; Bracco, Milan, Italy) injected at 5 mL/s, followed by an injection of 30 mL diluted contrast (contrast agent : saline = 3:7), and then 30 mL saline at 5 mL/s using a power injector (Envision CT; Medrad, Indianola, Pa) via an antecubital vein. The estimated volume CT dose index (CTDIvol) in milligrays (mGy) determined from the scanner's output parameters (kVp and mA per slice) was recorded for each patient. The product of CTDIvol and scanning length (dose–length product, mGy × cm) was calculated, and effective dose (ED, in millisieverts [mSv]) was estimated using a normalization factor for the adult chest (0.017 mSv × mGy^{-1} × cm^{-1}).

Measurement of outcome variables

Coronary CT angiograms were evaluated by two experienced cardiac radiologists (Y.J.K. and B.W.C., with 6 and 9 years of experience in cardiac CT, respectively) who were unaware of the clinical history of the patients. We selected two outcome variables: CAD, defined as presence of any plaque and CACS, measured using the scoring system previously described by Agatston *et al.*[25]

Plaque were defined as structures >1 mm^2 within and/or adjacent to the vessel lumen, which could be clearly distinguished from the vessel lumen and surrounding pericardial tissue. Plaque occupied by calcified tissue for more than 50% of the plaque area (density >130 Hounsfield Unit in native scans) were classified as calcified plaque. Plaque with $<50\%$ Ca were classified as mixed calcified plaque, and plaque without any Ca were classified as NCP.[26] The subjects with plaque were divided based on the plaque characteristics into two groups: individual with exclusively NCP and individual with calcified or mixed plaque. In addition, participants were further categorized based on the CACS in the following manner: CACS ≤ 100 and CACS > 100 as main categories; and CACS $= 0$, $0 <$ CACS ≤ 10, $10 <$ CACS ≤ 100, and CACS > 100 as subcategories. There were few individuals with CACS > 400 (3%); as a result, they were combined with those with CACS > 100.

Measurement of clinical variables and confounding variables

Medical history of hypertension, diabetes mellitus (DM), and smoking status were systematically acquired. Height, body weight, and blood pressure were measured during visits. Serum Ca, P, total cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose, glycated haemoglobin, blood urea nitrogen, and serum creatinine level were measured after a minimum of 12-h fasting period on the day of CT scan as the part of the clinical work-up. Creatinine was measured using rate-blanked and compensated Jaffé method with calibration for

automated systems (Roche Diagnostics, Basel, Switzerland). The level of kidney function was ascertained by estimated GFR calculated using the formula developed and validated in the MDRD study [27] as follows:

$$\mathrm{GFR\,(mL/min/1.73m^2)} = 186.3 \times (\mathrm{serumcreatinine[mg/dL]} - 1.154) \times (\mathrm{age[years]} - 0.203)(\times 0.742, \text{if female})$$

Serum C-reactive protein (CRP) was measured by latex agglutination with an auto-analyzer (Toshiba-200 FR, Tokyo, Japan). Diabetes was defined as treatment with hypoglycaemic agents or insulin, or fasting glucose ≥126 mg/dL. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or treatment with antihypertensive agents. Smoking history was considered present if patients currently smoked or smoked until 1 month before the study. Dyslipidaemia was defined as total cholesterol ≥240 mg/dL, LDL ≥130 mg/dL, HDL ≤40 mg/dL, TG ≥150 mg/dL and/or treatment with lipid lowering agents. Laboratory values for lipid parameters were obtained within 1 month prior to multi-slice CT examination.

Old age (≥60 years), male gender, dyslipidaemia, hypertension, and diabetes were considered as classical risk factors of cardiovascular diseases, and patients with 0 or 1 risk factors were classified as low-risk group, while those with 2 or more risk factors were classified as high-risk group.

Statistical analysis

Continuous variables are expressed as medians and interquartile range, whereas categorical variables are presented as absolute values and percentages. Differences between continuous variables were analysed by independent *t*-test or Mann-Whitney *U*-test and those between categorical variables by χ^2 test. Variables that showed significant relationships in previous studies and those with a $P < 0.3$ on univariate analysis were defined as confounding variables related to the dependent and independent variables. These included age, male gender, hypertension, DM, body mass index (BMI), MDRD GFR, and proteinuria. The covariate-adjusted odds ratios (OR) and their 95% confidence intervals (CI) for each dependent variable (CAD and CACS > 100) were calculated. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 18; SPSS, Chicago, IL, USA).

Results

The clinical characteristics of participants are shown in *Table 1*. The median age was 52 years, and 57% of the participants were male. Hypertension, DM, and dyslipidaemia was present in 30, 11, and 53% of the patients, respectively, and 21% were smokers. The medians and interquartile ranges of serum Ca, P, and CPP were 9.00 [8.70, 9.40] mg/dL, 3.70 [3.30, 4.10] mg/dL, and 33.3 [29.5, 37.8] mg^2/dL^2, respectively. The presence of plaque was confirmed by cCTA in 32.3% of all patients: when sub-classified, 25.2% showed calcified plaque, and 7.1% showed NCP. Coronary artery Ca score of 0 was seen in 73.9% of the patients and 8% had CACS > 100 (*Figure 1*).

As seen in *Table 2*, age, male gender, BMI, hypertension, diabetes, dyslipidaemia, and proteinuria demonstrated association with both CACS > 100 and the presence of CAD on univariate analysis. On the contrary, while serum Ca level was associated with both CACS > 100 and the presence of CAD (OR = 1.41, 95% CI: 1.23–1.61, $P < 0.001$; OR = 1.49, 95% CI: 1.38–1.61, $P < 0.001$, respectively), serum P level was negatively associated with the presence of CAD, and CPP was not significantly associated with either CACS > 100 or presence of CAD (*Table 2*). However, after adjusting for all confounding factors, including age, male gender, BMI, hypertension, diabetes, smoking status, dyslipidaemia and proteinuria, serum Ca, P, and CPP level were independent risk factors of CACS > 100, a marker of coronary atherosclerosis (OR: 1.22, 95% CI: 1.03–1.43, $P = 0.019$; OR: 1.28, 95% CI: 1.12–1.45, $P < 0.001$; and OR: 1.03, 95% CI: 1.02–1.05, $P < 0.001$, respectively), and the association was more strongly seen in higher quartiles. However, when compared with CACS > 100, the presence of CAD, another marker of coronary atherosclerosis, was more weakly associated with serum Ca, P, and CPP levels (OR: 1.19, 95% CI: 1.08–1.31, $P < 0.001$; OR: 1.05, 95% CI: 0.98–1.13, $P = 0.242$; and OR: 1.01, 95% CI: 1.00–1.02, $P = 0.034$, respectively; *Table 3*).

To elucidate the reason for the weak associations between serum Ca, P, and CPP, and the presence of CAD compared with those of CACS > 100, the presence of CAD was further divided into NCP and calcified or mixed plaque, and each group's association to serum Ca, P, and CPP was evaluated. Interestingly, serum Ca, P, and CPP are independent risk factors for calcified or mixed plaque (OR: 1.22, 95% CI: 1.09–1.35, $P < 0.001$; OR: 1.12, 95% CI: 1.03–1.22, $P = 0.006$; OR: 1.02, 95% CI: 1.01–1.03, $P < 0.001$, respectively), but their association with NCP was close to zero (OR: 1.07, 95% CI 0.91–1.25, $P = 0.423$; OR: 0.91, 95% CI: 0.80–1.04, $P = 0.155$; and OR: 1.00, 95% CI: 0.98–1.01, $P = 0.205$, respectively; *Figure 2*).

When the groups are sub-divided into various risk factors including age ≥60, gender, presence of symptom, hypertension, and diabetes, it can be seen that Ca, P, and CPP show associations with coronary atherosclerosis (see Supplementary material online, *Table S1*), although the significance of each factor may differ. Old age (≥60 years), male gender, dyslipidaemia, hypertension, and diabetes were considered as classical risk factors of cardiovascular diseases, and patients with 0 or 1 risk factors were classified as low-risk group, while those with 2 or more risk factors were classified as high-risk group. When subdivided into risk groups based on classical risk factors, the association between serum Ca, P, CPP, and coronary atherosclerosis was not seen in low-risk group, while it was seen in high-risk group. Upon further examination, Ca level was found to be significantly associated with CAD and calcified or mixed plaques in high-risk group—this association was not seen in NCPs of the same patient group (*Table 4*).

Discussion

Four clinical implications were demonstrated through this study. First, serum Ca, P, and CPP level are independent risk factors of coronary atherosclerosis, even in participants with near-normal kidney function. Second, this relationship is stronger in coronary calcification and is more pronounced in participants in the higher quartiles of serum Ca, P, and CPP. Third, the reason behind a relatively weak association between serum Ca, P, and CPP and the presence of CAD is inclusion of the group of NCP in CAD. The serum markers are independent risk factors for calcified or mixed plaque, similar to CACS. Lastly, while association between serum Ca, P, CPP, and coronary atherosclerosis was observed in high-risk group with 2 or more classical risk factors, no such association was found in low-risk group with 0 or 1 risk factor.

Table 1 Baseline characteristics

	CACS ≤ 100, (*n* = 6950)	CACS > 100, (*n* = 603)	*P*-value	No CAD (*n* = 5111)	CAD (*n* = 2442)	*P*-value
Age, years	50 (44, 58)	63 (57, 69)	<0.001	48 (43, 56)	58 (51, 65)	<0.001
Male	3864 (56)	446 (74)	<0.001	2630 (52)	1680 (69)	<0.001
Asymptomatic	4179 (60)	187 (31)	<0.001	3380 (66)	986 (40)	<0.001
BMI, kg/m^2	24.1 (22.2, 26.0)	24.4 (22.7, 26.5)	<0.001	23.9 (22.0, 25.8)	24.5 (22.8, 26.4)	<0.001
Hypertension	1951 (28)	301 (51)	<0.001	1227 (24)	1025 (43)	<0.001
DM	688 (10)	165 (28)	<0.001	359 (7)	494 (20)	<0.001
Smoking	1484 (22)	104 (18)	0.023	1108 (22)	480 (20)	0.070
Dyslipidaemia	3603 (52)	349 (59)	0.002	2491 (49)	1461 (61)	<0.001
T-chol, mg/dL	195 (172, 218)	190 (166, 213)	<0.001	194 (172, 217)	194 (170, 219)	0.619
HDL, mg/dL	52 (44, 61)	49 (42, 57)	<0.001	53 (45, 62)	49 (43, 58)	<0.001
LDL, mg/dL	111 (93, 133)	113 (93, 135)	0.593	110 (92, 130)	115 (96, 137)	<0.001
TG, mg/dL	113 (79, 164)	126 (91, 184)	<0.001	109 (75, 158)	128 (91, 182)	<0.001
High-sensitivity CRP	0.10 (0.01, 0.31)	0.29 (0.06, 0.82)	<0.001	0.01 (0.01, 0.24)	0.20 (0.01, 0.64)	<0.001
Serum Ca, mg/dL	9.00 (8.70, 9.40)	9.10 (8.80, 9.50)	<0.001	9.00 (8.60, 9.30)	9.10 (8.80, 9.50)	<0.001
Serum P, mg/dL	3.70 (3.30, 4.10)	3.70 (3.30, 4.10)	0.732	3.70 (3.30, 4.10)	3.70 (3.30, 4.10)	0.057
CPP, mg^2/dL^2	33.3 (29.4, 37.8)	33.8 (30.4, 37.8)	0.032	33.1 (29.4, 37.8)	33.6 (29.9, 37.8)	0.108
Serum chromium, mg/dL	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)	0.597	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)	0.018
Albumin creatinine ratio	6.10 (4.10, 9.80)	7.20 (4.98, 7.20)	<0.001	6.10 (6.00, 9.50)	6.80 (4.40, 12.6)	<0.001
GFR, mL/min/1.73 m^2	76 (69, 84)	76 (69, 86)	0.285	76 (69, 83)	76 (69, 85)	0.004
Proteinuria	420 (6.6)	62 (11.3)	<0.001	270 (5.8)	212 (9.6)	<0.001

Data are expressed as median [interquartile] or *n* (%). BMI, body mass index; CACS, coronary artery calcium score; CAD, coronary artery disease; Ca, calcium; CPP, calcium–phosphate product; DM, diabetes mellitus; GFR, glomerular filtration rate; HDL, high density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low density lipoprotein; P, phosphate; TG, triglyceride.

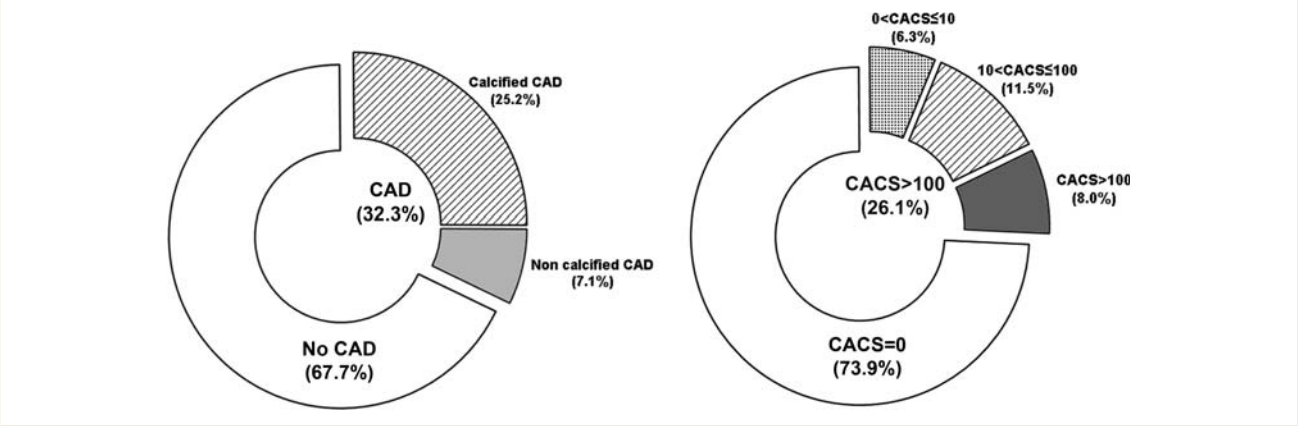

Figure 1 The distribution of coronary artery disease and coronary artery calcium score.

Serum calcium and phosphate induces coronary atherosclerosis by increasing coronary calcification in participants with near-normal kidney function

The relationship between serum Ca, P, and CPP and CVD was first studied in patients with renal failure. It is well-known that mineral regulation is impaired in patients with reduced kidney function, leading to Ca deposits that cause vascular calcification and increased risk of cardiovascular events. However, evidence of serum Ca and P levels affecting the incidence of cardiovascular events in patients with near-normal kidney function is lacking. Leifsson *et al.*[12] monitored 21 131 patients under 50 years old for 10.8 years, and have concluded that the mortality rate is higher in patients with high serum Ca, even within the normal range, and that cardiovascular disease was the major cause of mortality. In addition, Dihngra *et al.*[15] reported a 1.55-fold increased risk of CVD in patients in the highest serum P quartile among 3368

Table 2 Univariate correlations of calcium, phosphate, and calcium–phosphate product

	CACS		CAD	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age, years	1.13 (1.12–1.15)	<0.001	1.10 (1.09–1.11)	<0.001
Male gender	2.28 (1.89–2.75)	<0.001	2.08 (1.88–2.30)	<0.001
BMI, kg/m^2	1.05 (1.03–1.08)	<0.001	1.07 (1.05–1.09)	<0.001
Hypertension	2.64 (2.22–3.12)	<0.001	2.36 (2.13–2.62)	<0.001
DM	3.45 (2.83–4.19)	<0.001	3.36 (2.90–3.88)	<0.001
Current smoking	0.78 (0.62–0.97)	0.023	0.90 (0.79–1.01)	0.070
Dyslipidaemia	1.31 (1.11–1.56)	0.002	1.61 (1.46–1.78)	<0.001
Creatinine, mg/dL	1.07 (0.65–1.75)	0.791	1.28 (0.96–1.71)	0.090
High-sensitivity CRP, IU	1.06 (1.04–1.09)	<0.001	1.12 (1.08–1.16)	<0.001
Proteinuria	1.79 (1.35–2.37)	<0.001	1.73 (1.43–2.08)	<0.001
Ca, mg/dL	1.41 (1.23–1.61)	<0.001	1.49 (1.38–1.61)	<0.001
P, mg/dL	0.96 (0.87–1.07)	0.447	0.86 (0.83–0.94)	<0.001
CPP, mg^2/dL^2	1.01 (1.00–1.02)	0.291	1.00 (0.99–1.01)	0.690

CRP, c-reactive protein.

Table 3 Association between serum calcium, phosphate, and calcium–phosphate product with coronary atherosclerosis

	CACS>100		CAD	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Per 1 mg/dL of Ca	1.22 (1.03–1.43)	0.019	1.19 (1.08–1.31)	<0.001
By Ca quartile				
I (~8.6 mg/dL)	1		1	
II (8.7–8.9 mg/dL)	1.19 (0.86–1.64)	0.308	1.21 (1.01–1.44)	0.040
III (9.0–9.3 mg/dL)	1.68 (1.20–2.33)	0.002	1.33 (1.10–1.61)	0.003
IV (9.4 mg/dL)	1.43 (1.04–1.96)	0.029	1.35 (1.13–1.61)	0.001
Per 1 mg/dL of P	1.28 (1.12–1.45)	<0.001	1.05 (0.97–1.13)	0.242
By P quartile				
I (~3.2 mg/dL)	1		1	
II (3.3–3.6 mg/dL)	1.12 (0.83–1.52)	0.455	1.03 (0.86–1.24)	0.721
III (3.7~4.0 mg/dL)	1.41 (1.07–1.85)	0.015	1.28 (1.08–1.51)	0.004
IV (4.1 mg/dL)	1.76 (1.29–2.38)	<0.001	1.17 (0.98–1.40)	0.079
Per 1 mg^2/dL^2 of CPP	1.03 (1.02–1.05)	<0.001	1.01 (1.00–1.02)	0.045
By CPP quartile				
I (~29.4 mg^2/dL^2)	1		1	
II (29.5–33.2 mg^2/dL^2)	1.37 (1.02–1.85)	0.036	1.12 (0.94–1.33)	0.205
III (33.3–37.7 mg^2/dL^2)	1.68 (1.25–2.27)	0.001	1.31 (1.10–1.56)	0.002
IV (37.5 mg^2/dL^2)	1.92 (1.41–2.62)	<0.001	1.28 (1.07–1.53)	0.006

Adjusted for age, male gender, BMI, hypertension, DM, smoking, dyslipidaemia and proteinuria. OR, odds ratio; CI, confidence interval.

participants of the Framingham Offspring study over 16.1 years: Larsson *et al.*[28] showed that circulating Ca, P, and CPP are related to total, cardiovascular, and non-cardiovascular mortality rates in 2176 cohort participants over 29.8 years. Such longitudinal studies have consistently pointed to the association between serum Ca, P, and CPP and cardiovascular events in participants with near-normal kidney function, but its pathophysiology is still not clearly understood.

Roberts *et al.*[29] performed autopsies on 18 participants with chronic hypercalcaemia and found cardiac calcific deposits and

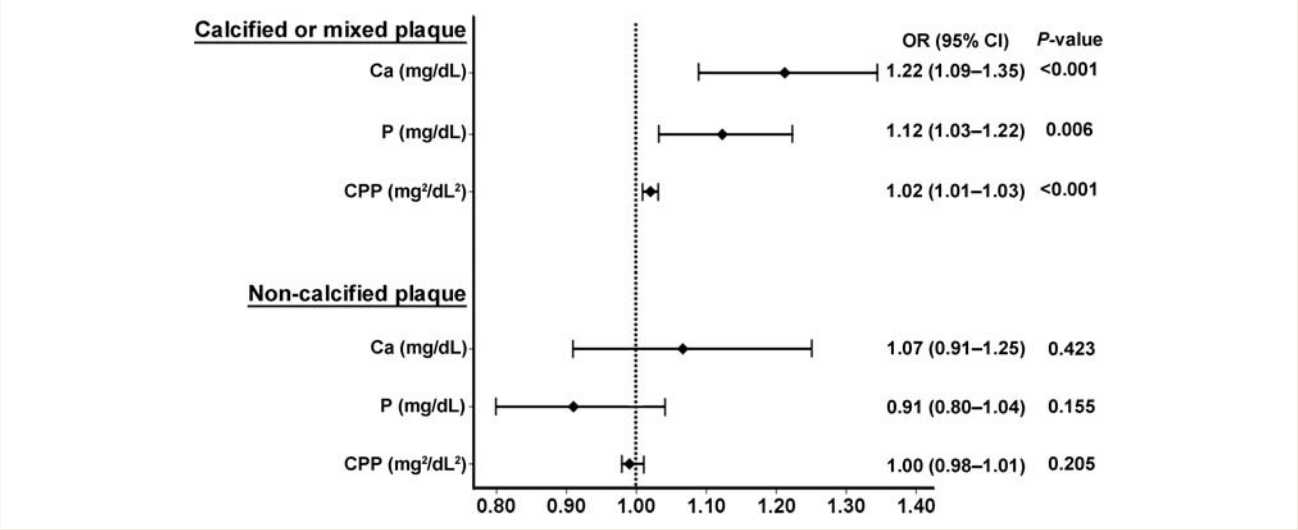

Figure 2 The association between calcium, phosphate, and calcium–phosphate product with the presence of coronary artery disease divided with calcified or mixed plaque and non-calcified plaque. The reason behind a relatively weak association between serum calcium, phosphate, and calcium–phosphate product; and the presence of coronary artery disease is inclusion of the group of non-calcified plaque in coronary artery disease.

Table 4 Association between calcium, phosphate, calcium–phosphate product, and coronary atherosclerosis based on classical risk factors of cardiovascular disease

	CACS > 100		CAD		Calcified or mixed plaque		NCP	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Low risk								
Per 1 mg/dL of Ca	1.25 (0.82–1.90)	0.309	1.21 (1.01–1.44)	0.035	1.26 (1.02–1.55)	0.032	1.05 (0.79–1.40)	0.727
Per 1 mg/dL of P	1.27 (0.94–1.72)	0.124	0.97 (0.85–1.11)	0.646	1.03 (0.88–1.21)	0.702	0.90 (0.73–1.10)	0.302
Per 1 mg^2/dL^2 of CPP	1.03 (0.10–1.07)	0.086	1.00 (0.99–1.02)	0.996	1.01 (0.99–1.03)	0.350	0.99 (0.96–1.01)	0.987
High risk								
Per 1 mg/dL of Ca	1.18 (0.99–1.41)	0.066	1.17 (1.04–1.31)	0.008	1.18 (1.05–1.33)	0.007	1.01 (0.84–1.22)	0.899
Per 1 mg/dL of P	1.27 (1.10–1.46)	0.001	1.08 (0.99–1.19)	0.093	1.16 (1.05–1.28)	0.004	0.89 (0.76–1.05)	0.156
Per 1 mg^2/dL^2 of CPP	1.03 (1.01–1.05)	<0.001	1.01 (1.00–1.02)	0.022	1.02 (1.01–1.03)	0.001	0.99 (0.97–1.01)	0.176

OR, odds ratio; CI, confidence interval.
Classical risk factor: old age (≥ 60 years), male gender, hypertension, diabetes, dyslipidaemia.
Low risk, zero or one risk factor; high risk, more than two risk factors.

progression of coronary atherosclerosis, demonstrating that hypercalcaemia in itself can be a risk for coronary atherosclerosis. In addition, increased cardiovascular mortality is seen in hyperthyroidism or chronic kidney disease, which is related to dysregulation of Ca metabolism, suggesting an association between Ca and cardiovascular mortality. Nonetheless, a causal mechanism between Ca and atherosclerosis has not been clearly found. Several articles assume that higher serum Ca affects the incidence of coronary atherosclerosis through its relationship with various metabolic diseases and other risk factors known to be related to the atherosclerotic process.[2,10]

Elevated serum P is also known to increase cardiovascular events and mortality in patients with CKD.[30] In addition, it is associated with mortality and recurrent events in CAD patients,[30] and is known to increase the incidence of CVD in communities of participants with near-normal kidney function.[15] The mechanism behind serum P inducing atherosclerosis has yet to be elucidated, but several possible explanations were suggested. First, high phosphorus levels were shown to inhibit 1,25-dihydroxyvitamin D synthesis.[31] Lower levels of 1,25-dihydroxyvitamin D are hypothesized to increase coronary calcification.[32] Additional studies are required to evaluate whether lower 1,25-dihydroxyvitamin D levels

contribute to greater calcification on coronary CT in individuals with higher serum P. Secondly, higher serum P levels may directly promote vascular injury. Investigators have reported that higher P levels increase the propensity of mineral deposition in vascular smooth muscle cells *in vitro*,[33] which can be partly explained by increased osteopontin expression or decreased fibroblast growth factor 23.[34,35] Serum P may also directly increase vascular calcification, especially when levels of CPP are high, as seen in patients with CKD[36] in most observational studies. However, this study showed that serum P is associated with increased coronary calcification also in patients with near-normal kidney function.

Serum calcium and phosphate do not increase the frequency of non-calcified plaque

Serum Ca, P, and CPP were independent risk factors for coronary atherosclerosis in patients with near-normal kidney function. However, while these serum markers show a consistently significant relationship with CACS, a surrogate marker of coronary atherosclerosis on CT, they are relatively weakly associated with the presence of CAD. We hypothesized that such discrepancy was because of the mechanism through which serum Ca, P, and CPP affect coronary atherosclerosis—namely, vascular calcification. Non-calcified plaque, which is included in CAD, may be thought of as a factor weakening the association between the serum markers and CAD. Therefore, the presence of CAD was further subdivided into NCP and calcified or mixed plaque, and similar to CACS, calcified or mixed plaque showed strong associations with serum Ca, P, and CPP, while NCP did not show such associations.

These findings may add to hypotheses explaining the conflicting results on the effect of Ca supplements on the incidence of cardiovascular events. Bolland *et al.*[16] concluded that Ca supplementation increases the incidence of cardiovascular events, especially myocardial infarction, whereas Lewis *et al.*[37] argued that Ca supplements do not increase mortality in patients with atherosclerotic cardiovascular events. Our study may explain such contradictory findings: while higher serum Ca, P, and CPP increase vascular calcification and coronary atherosclerosis progression, causing a modest increase in mortality, they are independent of NCPs, which are more strongly associated with acute coronary syndrome and increased mortality.

The association between calcium, phosphate, calcium–phosphate product, and coronary atherosclerosis based on classical risk factors of cardiovascular disease

We found that association between serum Ca, P, CPP, and coronary atherosclerosis is observed in high-risk group, but not in low-risk group. This implies that an isolated increase in Ca or P level does not lead to increased risk of coronary atherosclerosis across all patients. In other words, high Ca level may act as a trigger of coronary atherosclerosis under the presence of several risk factors. Indeed, Ca content was significantly greater among non-culprit lesions of patients with diabetes, who are at higher risk of cardiovascular diseases.[24,35] In diabetes, advanced glycation end products promote mineralization,[38] and the incidence of coronary atherosclerosis may increase in the setting of high Ca level. Similarly, in the patients with high risk of systemic inflammation such as patients with dyslipidaemia or patients older than 55 years, high Ca level may possibly accelerate vascular mineralization.[39] These results suggest that the presence of risk factors may affect the result of studies on association between Ca supplements and cardiovascular diseases, which has recently undergone controversies. Furthermore, the result also suggest that when determining the management of patients with osteoporosis, to whom Ca supplementation is essential, analysis of risk factors must be made in addition to simple serum Ca level. Prohibiting Ca supplemntation, based on the claim that it may increase Ca level, is thought to be inappropriate for osteoporotic patients with no cardiovascular risk factors.

Limitations

Several limitations to the study should be acknowledged. First, this is a retrospective, cross-sectional study performed at a single centre, and the patients had variable indications for MDCT, which makes it uncertain whether the results will be equally applicable to the general clinical practice. Second, parathormone levels and vitamin D levels, which could also be associated with adverse outcomes in renal failure, were not measured. However, the serum level of these hormones do not start to change until the estimated glomerular filtration rate falls below 60–70 mL/min/1.73 m^2, and we excluded all patients with such degree of renal insufficiency.[40] Third, we cannot exclude the possibility that our findings were influenced by dietary intake of P or Ca supplements, and that therefore, dietary habits or medications may confound the association between serum Ca and P and the results of coronary CT.

There are also several points unique to this study. First, this study examined patients of Asian ethnicity only. According to Nasir *et al.*,[41] the frequency of CACS $>$ 100 in Asian populations is low compared with Caucasians, African-Americans, and Hispanics. Compared with other similar studies performed in Western nations, this study is novel in that only participants of Asian ethnicity were studied. In addition, this study enrolled nearly 8000 patients exclusively with near-normal kidney function and obtained large-scale CT data: the finding that serum Ca, P, and CPP were independent risk factors of coronary atherosclerosis confirmed by coronary CT scan is meaningful. Furthermore, within coronary atherosclerosis, Ca, P, and CPP affect relatively stable calcified or mixed plaques only, and are unrelated to NCP. Such findings may contribute to additional hypotheses to explain the conflicting results of studies examining the effect of Ca supplements on cardiovascular events.

Whether a strict control of serum Ca or P level is necessary for patients with normal kidney function requires a careful approach. Previous large-scale cohort studies have also demonstrated associations between serum Ca and P and CVD[12,15]; however, unlike in CKD patients, there is no clear evidence suggesting benefits of serum mineral control in those with normal kidney function. Furthermore, there is no established target range for serum mineral levels, and evidence demonstrating hazards of such supplements

is lacking compared with their clear benefits on osteoporosis. For further studies, a longitudinal study monitoring the change in the atherosclerosis of the patients with high serum Ca and P using cCTA seems plausible, as well as a follow-up cohort study observing the relationship serum Ca and P distribution and the differences between initial and follow-up cCTA. In addition, these studies must analyse the effect of Ca supplementation on cardiovascular disease after stratifying the risk factors of the disease.

Conclusions

Elevated serum levels of Ca, P, and CPP are significantly associated with the presence of calcified coronary atherosclerotic plaque. It is unclear if there is a causal relationship. This relationship is thought to contribute to vascular calcification, but is less closely associated with the formation of NCP.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Funding

Technology Innovation Program (Industrial Strategic Technology Development Program, 10038419, Intelligent Image Diagnosis and Therapy-support System) granted from Ministry of Knowledge Economy (MKE, Korea)

Conflict of interest: none declared.

References

1. Palmer M, Adami HO, Bergstrom R, Jakobsson S, Akerstrom G, Ljunghall S. Survival and renal function in untreated hypercalcaemia. Population-based cohort study with 14 years of follow-up. *Lancet* 1987;**1**:59–62.
2. Hagstrom E, Hellman P, Lundgren E, Lind L, Arnlov J. Serum calcium is independently associated with insulin sensitivity measured with euglycaemic-hyperinsulinaemic clamp in a community-based cohort. *Diabetologia* 2007;**50**:317–324.
3. Hagstrom E, Lundgren E, Mallmin H, Rastad J, Hellman P. Positive effect of parathyroidectomy on bone mineral density in mild asymptomatic primary hyperparathyroidism. *J Intern Med* 2006;**259**:191–198.
4. Block GA, Hulbert-Shearon TE, Levin NW, Port FK. Association of serum phosphorus and calcium × phosphate product with mortality risk in chronic hemodialysis patients: a national study. *Am J Kidney Dis* 1998;**31**:607–617.
5. Ganesh SK, Stack AG, Levin NW, Hulbert-Shearon T, Port FK. Association of elevated serum PO(4), Ca × PO(4) product, and parathyroid hormone with cardiac mortality risk in chronic hemodialysis patients. *J Am Soc Nephrol* 2001;**12**:2131–2138.
6. Stevens LA, Djurdjev O, Cardew S, Cameron EC, Levin A. Calcium, phosphate, and parathyroid hormone levels in combination and as a function of dialysis duration predict mortality: evidence for the complexity of the association between mineral metabolism and outcomes. *J Am Soc Nephrol* 2004;**15**:770–779.
7. Tomiyama C, Higa A, Dalboni MA, Cendoroglo M, Draibe SA, Cuppari L, Carvalho AB, Neto EM, Canziani ME. The impact of traditional and non-traditional risk factors on coronary calcification in pre-dialysis patients. *Nephrol Dial Transplant* 2006;**21**:2464–2471.
8. Goodman WG, Goldin J, Kuizon BD, Yoon C, Gales B, Sider D, Wang Y, Chung J, Emerick A, Greaser L, Elashoff RM, Salusky IB. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med* 2000;**342**:1478–1483.
9. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM. Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. *J Am Soc Nephrol* 2004;**15**:2208–2218.
10. Lind L, Jakobsson S, Lithell H, Wengle B, Ljunghall S. Relation of serum calcium concentration to metabolic risk factors for cardiovascular disease. *BMJ* 1988;**297**:960–963.
11. Jorde R, Sundsfjord J, Fitzgerald P, Bonaa KH. Serum calcium and cardiovascular risk factors and diseases: the Tromso study. *Hypertension* 1999;**34**:484–490.
12. Leifsson BG, Ahren B. Serum calcium and survival in a large health screening program. *J Clin Endocrinol Metab* 1996;**81**:2149–2153.
13. Onufrak SJ, Bellasi A, Shaw LJ, Herzog CA, Cardarelli F, Wilson PW, Vaccarino V, Raggi P. Phosphorus levels are associated with subclinical atherosclerosis in the general population. *Atherosclerosis* 2008;**199**:424–431.
14. Ix JH, De Boer IH, Peralta CA, Adeney KL, Duprez DA, Jenny NS, Siscovick DS, Kestenbaum BR. Serum phosphorus concentrations and arterial stiffness among individuals with normal kidney function to moderate kidney disease in MESA. *Clin J Am Soc Nephrol* 2009;**4**:609–615.
15. Dhingra R, Sullivan LM, Fox CS, Wang TJ, D'Agostino RB Jr., Gaziano JM, Vasan RS. Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. *Arch Intern Med* 2007;**167**:879–885.
16. Bolland MJ, Grey A, Avenell A, Gamble GD, Reid IR. Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. *BMJ* 2011;**342**:d2040.
17. Budoff MJ, Shaw LJ, Liu ST, Weinstein SR, Mosler TP, Tseng PH, Flores FR, Callister TQ, Raggi P, Berman DS. Long-term prognosis associated with coronary calcification: observations from a registry of 25,253 patients. *J Am Coll Cardiol* 2007;**49**:1860–1870.
18. Detrano R, Guerci AD, Carr JJ, Bild DE, Burke G, Folsom AR, Liu K, Shea S, Szklo M, Bluemke DA, O'Leary DH, Tracy R, Watson K, Wong ND, Kronmal RA. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. *N Engl J Med* 2008;**358**:1336–1345.
19. Cho I, Min HS, Chun EJ, Park SK, Choi Y, Blumenthal RS, Rivera JJ, Nasir K, Kim YJ, Sohn DW, Oh BH, Park YB, Chang HJ. Coronary atherosclerosis detected by coronary CT angiography in asymptomatic subjects with early chronic kidney disease. *Atherosclerosis* 2010;**208**:406–411.
20. Hoffmann MH, Shi H, Schmitz BL, Schmid FT, Lieberknecht M, Schulze R, Ludwig B, Kroschel U, Jahnke N, Haerer W, Brambs HJ, Aschoff AJ. Noninvasive coronary angiography with multislice computed tomography. *JAMA* 2005;**293**:2471–2478.
21. Lee S, Choi EK, Chang HJ, Kim CH, Seo WW, Park JJ, Sang Il C, Chun EJ, Chang SA, Kim HK, Kim YJ, Koo BK, Choi DJ, Oh B. Subclinical coronary artery disease as detected by coronary computed tomography angiography in an asymptomatic population. *Korean Circ J* 2010;**40**:434–441.
22. Budoff MJ, Dowe D, Jollis JG, Gitter M, Sutherland J, Halamert E, Scherer M, Bellinger R, Martin A, Benton R, Delago A, Min JK. Diagnostic performance of 64-multidetector row coronary computed tomographic angiography for evaluation of coronary artery stenosis in individuals without known coronary artery disease: results from the prospective multicenter ACCURACY (Assessment by Coronary Computed Tomographic Angiography of Individuals Undergoing Invasive Coronary Angiography) trial. *J Am Coll Cardiol* 2008;**52**:1724–1732.
23. Miller JM, Rochitte CE, Dewey M, Arbab-Zadeh A, Niinuma H, Gottlieb I, Paul N, Clouse ME, Shapiro EP, Hoe J, Lardo AC, Bush DE, de Roos A, Cox C, Brinker J, Lima JA. Diagnostic performance of coronary angiography by 64-row CT. *N Engl J Med* 2008;**359**:2324–2336.
24. Kim JH, Kim DJ, Jang HC, Choi SH. Epidemiology of micro- and macrovascular complications of type 2 diabetes in Korea. *Diabetes Metab J* 2011;**35**:571–577.
25. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol* 1990;**15**:827–832.
26. Leber AW, Becker A, Knez A, von Ziegler F, Sirol M, Nikolaou K, Ohnesorge B, Fayad ZA, Becker CR, Reiser M, Steinbeck G, Boekstegers P. Accuracy of 64-slice computed tomography to classify and quantify plaque volumes in the proximal coronary system: a comparative study using intravascular ultrasound. *J Am Coll Cardiol* 2006;**47**:672–677.
27. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, Hogg RJ, Perrone RD, Lau J, Eknoyan G, National Kidney F. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Intern Med* 2003;**139**:137–147.
28. Larsson TE, Olauson H, Hagstrom E, Ingelsson E, Arnlov J, Lind L, Sundstrom J. Conjoint effects of serum calcium and phosphate on risk of total, cardiovascular, and noncardiovascular mortality in the community. *Arterioscler Thromb Vasc Biol* 2010;**30**:333–339.
29. Roberts WC, Waller BF. Effect of chronic hypercalcemia on the heart. An analysis of 18 necropsy patients. *Am J Med* 1981;**71**:371–384.
30. Kestenbaum B, Sampson JN, Rudser KD, Patterson DJ, Seliger SL, Young B, Sherrard DJ, Andress DL. Serum phosphate levels and mortality risk among people with chronic kidney disease. *J Am Soc Nephrol* 2005;**16**:520–528.
31. Portale AA, Halloran BP, Morris RC Jr. Physiologic regulation of the serum concentration of 1,25-dihydroxyvitamin D by phosphorus in normal men. *J Clin Invest* 1989;**83**:1494–1499.
32. Watson KE, Abrolat ML, Malone LL, Hoeg JM, Doherty T, Detrano R, Demer LL. Active serum vitamin D levels are inversely correlated with coronary calcification. *Circulation* 1997;**96**:1755–1760.

33. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, Morii H, Giachelli CM. Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res* 2000;**87**:E10–E17.
34. Giachelli CM, Speer MY, Li X, Rajachar RM, Yang H. Regulation of vascular calcification: roles of phosphate and osteopontin. *Circ Res* 2005;**96**:717–722.
35. Kuro-o M. Klotho and the aging process. *Korean J Intern Med* 2011;**26**:113–122.
36. Oh J, Wunsch R, Turzer M, Bahner M, Raggi P, Querfeld U, Mehls O, Schaefer F. Advanced coronary and carotid arteriopathy in young adults with childhood-onset chronic renal failure. *Circulation* 2002;**106**:100–105.
37. Lewis JR, Calver J, Zhu K, Flicker L, Prince RL. Calcium supplementation and the risks of atherosclerotic vascular disease in older women: results of a 5-year RCT and a 4.5-year follow-up. *J Bone Miner Res* 2011;**26**:35–41.
38. Yamagishi S, Fujimori H, Yonekura H, Tanaka N, Yamamoto H. Advanced glycation endproducts accelerate calcification in microvascular pericytes. *Biochem Biophys Res Commun* 1999;**258**:353–357.
39. Raaz-Schrauder D, Klinghammer L, Baum C, Frank T, Lewczuk P, Achenbach S, Cicha I, Stumpf C, Wiltfang J, Kornhuber J, Daniel WG, Garlichs CD. Association of systemic inflammation markers with the presence and extent of coronary artery calcification. *Cytokine* 2012;**57**:251–257.
40. Levin A, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA, Andress DL. Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. *Kidney Int* 2007;**71**:31–38.
41. Nasir K, Shaw LJ, Liu ST, Weinstein SR, Mosler TR, Flores PR, Flores FR, Raggi P, Berman DS, Blumenthal RS, Budoff MJ. Ethnic differences in the prognostic value of coronary artery calcification for all-cause mortality. *J Am Coll Cardiol* 2007;**50**:953–960.