Association of Bone Activity, Calcium Load, Aortic Stiffness, and Calcifications in ESRD

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ABSTRACT

An inverse relationship between arterial calcifications and bone activity has been documented in patients with ESRD. Calcium overload is associated with arterial calcification, which is associated with arterial stiffening. Whether bone activity interacts with calcium load, aortic stiffness, or arterial calcification is unknown. This study assessed the impact of bone activity on the relationships between the dosage of calcium-containing phosphate binders and aortic stiffness (measured by pulse wave velocity) or abdominal aorta calcification score. Aortic stiffness and calcification were both positively associated with calcium load and negatively associated with bone activity. A significant interaction was found between dosage of calcium-containing phosphate binders and bone activity such that calcium load had a significantly greater influence on aortic calcifications and stiffening in the presence of adynamic bone disease. Independent of any other factor, including dosage of calcium-containing phosphate binders, adynamic bone was associated with greater aortic stiffening, suggesting cross-talk between the bone and arterial walls.

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Arterial calcifications (AC) are a common complication of chronic kidney (CKD) and ESRD. [1–4] In the general population and patients with ESRD, the extents of AC were associated with aortic stiffening and were predictive of subsequent cardiovascular disease and mortality beyond established conventional risk factors.[5–8] The mechanisms responsible are complex, because AC is a regulated process with plasma constituents maintaining minerals in solution and inhibiting their deposition in tissues.[9] An inverse relationship between AC and bone density was documented in general populations,[10,11] and in patients with ESRD, the extent of AC was associated with low bone activity and adynamic bone disease (ABD).[12] Disturbances in calcium (Ca) and phosphate (PO_4) metabolism are associated with uremic bone disease, and the results of several studies indicated that Ca overload is associated with AC presence and progression.[4,13] All of these observations strongly suggest complex interplay among bone activity, Ca load, and AC development, but whether the bone activity is involved in Ca load influence on AC is unknown. Aortic stiffness, a predictor of all-cause and cardiovascular mortality in ESRD, is associated with aortic calcifications (AoC), but whether it is influenced by Ca load and bone activity is not known. This study was designed to evaluate the relationships and interaction between bone activity, as assessed by bone histomorphometry, and

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the use of Ca-containing PO_4 binders with the extent of abdominal aortic AoC and aortic stiffness assessed by pulse wave velocity (PWV) in patients who had ESRD and were undergoing chronic hemodialysis.

RESULTS

Characteristics of the Study Population

The distribution of the percentage of double tetracycline labeling is shown in Figure 1. Aortic, demographic, clinical, biochemical, and bone histomorphometry characteristics as a function of bone activity are summarized in Table 1. Patients with ABD were older and characterized by significantly higher abdominal aortic calcification scores (AoCS) and aortic PWV, lower serum albumin, higher C-reactive protein, and lower parathormone (PTH). Serum PO_4 levels were comparable, but Ca-containing PO_4 binder dosages were higher for patients with ABD, and more of them had surgical parathyroidectomy (PTX)-related hypoparathyroidism. Primary kidney diseases were comparably distributed in the two groups. All bone activity and remodeling indexes were significantly lower for patients with ABD, whereas their trabecular and osteoid volumes did not differ from those with active bone.

Table 2 gives the characteristics of AoC-negative and AoC-positive patients with ESRD. AoC-negative patients were younger, had shorter hemodialysis vintage, and smoked less. They had significantly higher bone activity, less pronounced microinflammation, and lower Ca-containing PO_4 binder doses. Aortic PWV was significantly lower in AoC-negative than in AoC-positive patients.

Bone Activity, "Ca Load," and Aortic Changes

The univariate correlation between the daily elemental Ca dosage provided by $CaCO_3$ and AoCS or aortic PWV according to bone activity is shown in Figure 2. Significant positive correlations between Ca dosage and AoCS or aortic PWV were observed for patients with ABD. Although biochemistry parameters, such as PTH, differed significantly between the two groups, Figure 3 shows that values overlapped between the two groups and a large proportion of patients with Kidney Disease Outcomes Quality Initiative (KDOQI)-recommended PTH levels (150 to 300 pg/ml) had ABD. The extent of overlapping was minimized when analyzed as a function of bone dynamics (Figure 3).

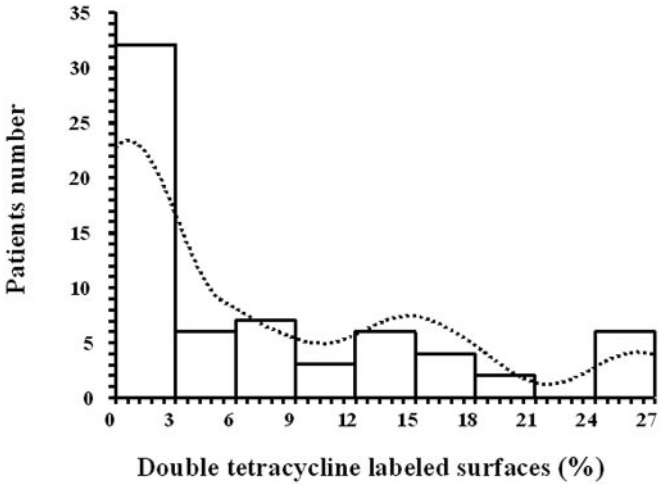

Figure 1. Histogram showing the number of patients with double tetracycline–labeled surface values.

Analyses of variance data (Table 3) showed significant interaction between Ca load (elemental Ca in g/d), bone activity (ABD, 0; active bone, 1), and AoCS ($P = 0.002$) or aortic PWV ($P = 0.003$) in the entire population. The adjusted multivariate analyses of variables associated with AoCS or aortic PWV for the entire population of 66 patients with ESRD are reported in Table 4. AoCS was negatively correlated with serum albumin, bone activity (ABD, 0; active bone, 1), gender (male, 0; female, 1), and Ca dosage*bone activity interaction. Positive correlations were observed with $CaCO_3$ dosage, age, and vintage. Double tetracycline–labeled surfaces were negatively associated with age ($P = 0.04$) and positively associated with resorption surface ($P = 0.03$) and osteoblastic surface ($P < 0.0001$) but not with aluminum (Al)-stained surfaces ($P = 0.3$). Aortic PWV was positively associated with age and Ca dosage and negatively with bone activity (ABD, 0; active bone, 1) and Ca-dosage*bone activity interaction (Table 4). In multivariate analysis, the association with BP was NS. A significant inverse correlation existed between aortic PWV and double tetracycline–labeled surface (Figure 4).

DISCUSSION

The results of several previous cross-sectional and longitudinal studies indicated that vascular calcifications were associated with the use of Ca-containing PO_4 binders, but the interaction between bone activity and Ca load on PWV and vascular calcifications were not analyzed.[1,2,4] The results of this study highlighted the complexity of the relationship between Ca load and aortic stiffness or AoCS, with bone turnover playing an important role. In patients with ESRD, aortic PWV and AoCS depended significantly on $CaCO_3$ dosage and bone activity, and the dosage of Ca-containing PO_4 binders and bone activity interacted significantly. The positive associations between Ca load and PWV or AoCS were stronger in patients with ABD, indicating that the presence of ABD conferred significantly greater influence of Ca load on aortic calcifications and stiffening. The presence of an active bone was associated with lower aortic stiffness and better aortic capacitive function.

ABD was associated with aging, microinflammation and hypoalbuminemia, and more frequent surgical PTX-related hypoparathyroidism. Although the role of PTX is more specific to patients with ESRD, the associations of age or microinflammation with bone density and activity have been observed in nonuremic populations. The results of several studies showed

Table 1. Aortic, clinical, biochemical, and bone characteristics of the 66 patients with ESRD[a]

Variable	Active Bone (n = 33)	Adynamic Bone (n = 33)	P
AoCS (mean ± SEM)	3.60 ± 0.80	12.00 ± 1.10	<0.0001
Aortic PWV (m/s; mean ± SEM)	9.43 ± 0.35	12.52 ± 0.38	<0.0001
Age (yr; mean ± SEM)	43.00 ± 2.60	53.60 ± 1.60	<0.0010
Gender (male/female)	16/17	17/16	NS
Smoking (pack-years; median [95% CI])	5.00 (0.00 to 16.00)	3.50 (0.00 to 9.00)	NS
Vintage (mo; mean ± SEM)	80.00 ± 8.50	90.00 ± 11.40	NS
SBP (mmHg; mean ± SEM)	154.00 ± 4.20	158.50 ± 3.60	NS
DBP (mmHg; mean ± SEM)	85.60 ± 2.40	84.20 ± 2.30	NS
Antihypertensive drugs (no/yes)	11/22	11/22	NS
Total cholesterol (mmol/L; mean ± SEM)	5.07 ± 0.25	5.21 ± 0.18	NS
Serum albumin (g/L; mean ± SEM)	39.7 ± 0.40	37.5 ± 0.40	<0.0010
CRP (mg/L; median [95% CI])	3.00 (2.00 to 5.00)	9.00 (7.00 to 11.00)	<0.0010
Serum Ca (mmol/L; mean ± SEM)	2.43 ± 0.03	2.43 ± 0.03	NS
Ionized Ca (mmol/L; mean ± SEM)	1.23 ± 0.01	1.20 ± 0.01	NS
Serum PO_4 (mmol/L; mean ± SEM)	1.92 ± 0.06	2.03 ± 0.07	NS
Ca*PO_4 ($mmol^2/L^2$; mean ± SEM)	4.67 ± 0.15	4.95 ± 0.19	NS
PTH (pg/ml; median [95% CI])	420.00 (327.00 to 549.00)	142.00 (57.00 to 570.00)	<0.0001
Serum Al (μmol/L; mean ± SEM)	1.28 ± 0.14	1.60 ± 0.17	NS
Deferoxamine test (ΔAl μmol/L; mean ± SEM)	2.03 ± 0.28	2.60 ± 0.28	NS
$CaCO_3$ (g elemental Ca/d; mean ± SEM)	1.47 ± 0.20	1.97 ± 0.14	0.0200
1α-OH-D_3 (μg/d; median [95% CI])	0.25 (0.00 to 0.25)	0.00 (0.00 to 0.25)	NS
Resorption surfaces (%; median [95% CI])	2.83 (2.18 to 3.47)	0.19 (0.00 to 0.50)	<0.0001
Osteoclasts/mm^2 (median [95% CI])	2.14 (1.31 to 2.69)	0.18 (0.09 to 0.40)	<0.0001
Osteoblastic surfaces (%; median [95% CI])	13.20 (10.50 to 15.00)	1.17 (0.42 to 2.12)	<0.0001
Double tetracycline–labeled surfaces (%; median [95% CI])	13.50 (8.00 to 16.0)	0.00 (0.00 to 0.00)	<0.0001
Trabecular volume (%; median [95% CI])	21.2 (15.3 to 23.8)	19.5 (15.4 to 29.1)	NS
Osteoid surface (%; median [95% CI])	53.5 (44.0 to 64.0)	43.5 (33.0 to 58.0)	NS
Al-stained surfaces (%; median [95% CI])	6.0 (0.0 to 23.0)	46.0 (17.0 to 75.0)	<0.0100
Post-PTX hypoparathyroidism (yes/no)	0/33	8/25	0.0020
Glomerulonephritis (n)	15	11	NS
CPNPh, interstitial nephritis (n)	11	10	NS
Polycystic kidney disease (n)	3	8	NS
Nephroangiosclerosis (n)	3	2	NS
Diabetes (n)	1	2	NS

[a]Reference values: Resorption surfaces (%): 0.4 ± 0.3; osteoclasts/mm^2: 0.15 ± 0.05; osteoblastic surfaces (%): 4 ± 2; trabecular volume (%): 15 to 22; osteoid surface (%): 15 ± 5. CI, confidence interval; CPNPh, chronic pyelonephritis; CRP, C-reactive protein; DBP, diastolic BP; SBP, systolic BP.

that chronic inflammation is associated with bone loss and heightened fracture risk[14,15] and cardiovascular calcifications.[16,17]

Although the serum PO_4 level did not differ as a function of bone activity, for maintaining similar PO_4 levels, patients with ABD received higher dosages of Ca-containing PO_4 binders. As shown in Table 3, AoCS was significantly associated with Ca load and bone activity, with a significant Ca dosage*bone activity interaction. In multivariate analyses, the β coefficient of the interaction term was negative, indicating that the correlation between Ca load and AoCS differed according to bone activity and was stronger in patients with ABD, indicating that the presence of ABD conferred significantly greater influence of Ca load. Vintage was longer for patients with ABD, and a higher percentage of those patients had been treated with Al-containing PO_4 binders in the past. Although the prescription of Al-containing PO_4 binders was stopped several years before the bone biopsy, patients with ABD still had higher bone Al-stained surfaces but comparable serum Al concentrations and deferoxamine test. Several clinical and experimental studies demonstrated that Al reduced circulating PTH by decreasing its synthesis and release[18–20] and could induce ABD. Bone Al deposition rises after subtotal PTX, and it is likely that low PTH and low remodeling could facilitate bone Al deposition.[21]

In this study, the extent of double tetracycline labeling was negatively correlated with age and positively with osteoclastic resorption and osteoblastic surfaces but not directly with serum Al, deferoxamine-induced serum Al changes, and Al-stained surfaces. A significant negative, albeit weak, correlation was observed between Al-stained surfaces and osteoblastic surfaces, suggesting that even an ancient Al overload could still influence bone activity. The results of multivariate analyses indicated that, after adjustment for significant covariates including hemodialysis vintage, Al-overload indices were not

Table 2. Characteristics of the 66 patients with ESRD as a function of AoC status

Variable	AoC Negative (n = 18)	AoC Positive (n = 48)	P
Age (yr; mean ± SEM)	32.80 ± 2.50	53.60 ± 1.40	<0.00010
Aortic PWV (m/s; mean ± SEM)	8.48 ± 0.38	11.63 ± 0.35	<0.00010
Smoking (pack-years; median [95% CI])	0.00 (0.00 to 5.00)	5.00 (1.00 to 15.00)	<0.01000
Vintage (mo; mean ± SEM)	59.00 ± 7.90	94.00 ± 9.10	0.01000
SBP (mmHg; mean ± SEM)	151.00 ± 6.20	158.00 ± 3.10	NS
DBP (mmHg; mean ± SEM)	86.00 ± 3.80	86.00 ± 1.80	NS
Total cholesterol (mmol/L; mean ± SEM)	4.87 ± 0.30	5.18 ± 0.16	NS
Serum albumin (g/L; mean ± SEM)	40.80 ± 0.40	37.90 ± 0.30	<0.00010
CRP (mg/L)	2.00 (1.00 to 3.00)	8.00 (7.00 to 10.00)	<0.00010
Serum Ca (mmol/L; mean ± SEM)	2.40 ± 0.04	2.43 ± 0.02	NS
Ionized Ca (mmol/L; mean ± SEM)	1.23 ± 0.01	1.21 ± 0.01	NS
Serum PO_4 (mmol/L; mean ± SEM)	1.81 ± 0.06	2.06 ± 0.06	<0.01000
Ca*PO_4 ($mmol^2/L^2$; mean ± SEM)	4.35 ± 0.17	5.00 ± 0.15	<0.01000
PTH (pg/ml; median [95% CI])	411.00 (229.00 to 670.00)	206.00 (160.00 to 291.00)	<0.00001
Serum Al (μ mol/L; mean ± SEM)	0.96 ± 0.13	1.62 ± 0.14	<0.01000
Deferoxamine test (ΔAl μ mol/L; mean ± SEM)	1.48 ± 0.26	2.70 ± 0.24	<0.01000
$CaCO_3$ (g of elemental Ca/d; mean ± SEM)	1.270 ± 0.300	1.900 ± 0.132	0.02000
1α-OH-D_3 (μ g/wk; median [95% CI])	0.25 (0.00 to 0.50)	0.00 (0.00 to 0.25)	NS
Resorption surfaces (%; median [95% CI])	2.56 (1.71 to 3.85)	0.68 (0.27 to 1.40)	<0.00100
Osteoclasts/mm^2 (median [95% CI])	1.70 (1.05 to 2.70)	0.47 (0.28 to 1.00)	<0.00100
Osteoblastic surfaces (%; median [95% CI])	13.00 (10.20 to 15.20)	3.40 (1.20 to 7.30)	<0.00100
Double tetracycline–labeled surfaces (%; median [95% CI])	15.00 (9.00 to 18.50)	1.30 (0.00 to 3.20)	<0.00100
Al-stained surfaces (%; median [95% CI])	0.00 (0.00 to 7.50)	28.50 (17.00 to 50.30)	<0.01000
Post-PTX hypoparathyroidism (yes/no) (n)	0/18	8/40	<0.01000

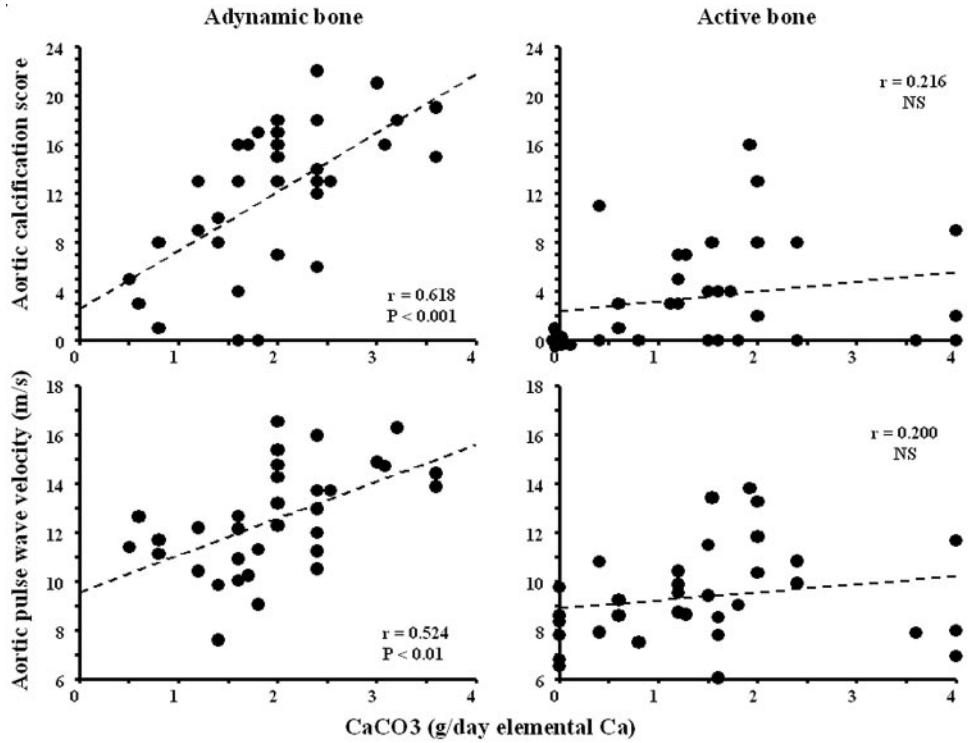

Figure 2. Correlations between daily $CaCO_3$ dosage expressed in grams of elemental Ca and AoCS or aortic PWV for patients with active bone and those with ABD.

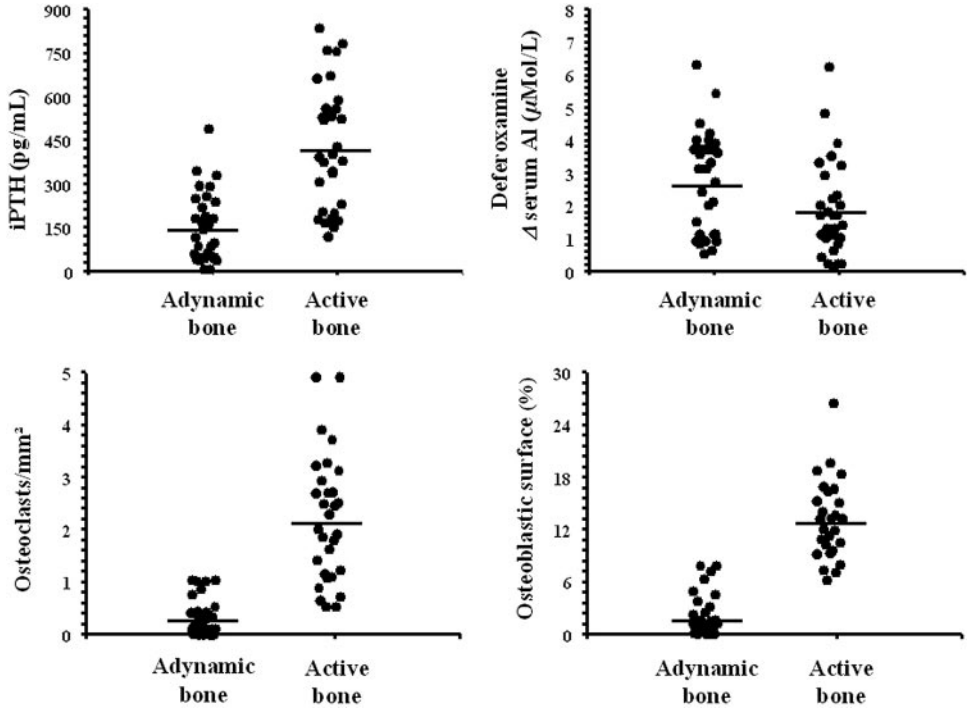

Figure 3. Biochemistry and bone histomorphometry differences between patients with active bone and those with ABD.

Table 3. Analysis of covariance with abdominal AoCS or aortic PWV[a]

Term	F ratio	*P*
Abdominal AoCS		
X_1 vintage (mo)	15.79	0.0003
X_2 age (yr)	52.90	<0.0001
A $CaCO_3$ (g elemental Ca/d)	10.57	<0.0001
B bone activity (0, ABD; 1, active bone)	6.50	0.0200
AB interaction (bone status*$CaCO_3$)	3.28	0.0020
Aortic PWV (m/s)		
X_1 age (yr)	27.65	<0.0001
X_2 mean BP (mmHg)	6.34	0.0100
A $CaCO_3$ (g elemental Ca/d)	7.37	<0.0001
B bone activity (0, ABD; 1, active bone)	9.44	0.0400
AB interaction (bone status*$CaCO_3$)	4.64	0.0030

[a]A, abdominal AoCS; B, aortic PWV; X, covariate.

Table 4. Multivariate analysis of variables associated with abdominal AoCS or aortic PWV ($n = 66$)

Variable	β Coefficient	*T*	*P*
Abdominal AoCS[a]			
intercept	16.100	1.856	0.0700
serum albumin (g/L)	−0.530	−2.661	0.0100
gender (0, male; 1, female)	−2.330	−3.188	0.0020
bone activity (0, ABD; 1, active bone)	−2.820	−2.801	0.0070
bone/Ca interaction	−0.130	−2.075	0.0400
$CaCO_3$ (g elemental Ca/d)	2.710	5.573	<0.0001
age (yr)	0.130	3.639	<0.0010
vintage (mo)	0.020	3.486	<0.0010
Aortic PWV (m/s)[b]			
intercept	4.540	2.911	0.0050
age (yr)	0.070	4.410	<0.0001
mean BP (mmHg)	0.020	1.721	0.0900
$CaCO_3$ (g elemental Ca/d)	1.010	4.389	<0.0001
bone activity (0, ABD; 1, active bone)	−1.130	−2.244	0.0300
bone/Ca interaction	−0.100	−2.346	0.0200

[a]Adjusted r^2 for the model 0.8258; F ratio 45.01; $P < 0.00001$.
[b]Adjusted r^2 for the model 0.7020; F ratio 31.61; $P < 0.00001$.

correlated with AoCS or aortic PWV (Table 4). The data published to date do not favor a possible association between Al and aortic stiffness or AoCS, because elastin calcifications and elastolysis by matrix metalloproteinases are prevented by Al chloride.[22,23] Furthermore, several patients with active bone, including those without AoC, had received Al-containing PO_4 binders (Figure 3).

The principal novelty of this study concerns the relationships between Ca load, bone activity, and aortic stiffness. The most important determinants of aortic stiffness are age, BP, and composition of arterial walls. The influence of these factors on aortic PWV are shown in Table 4, and the relationship between abdominal AoCS and PWV confirmed previous observations.[24] The results of several studies documented an inverse relationship between arterial stiffness and vertebral bone density[25] or osteoporosis.[26,27] These associations could, for the most part, be related to common effects of factors influencing both PWV and bone density, such as the aging process, microinflammation, or smoking.[28] Aortic PWV was significantly as-

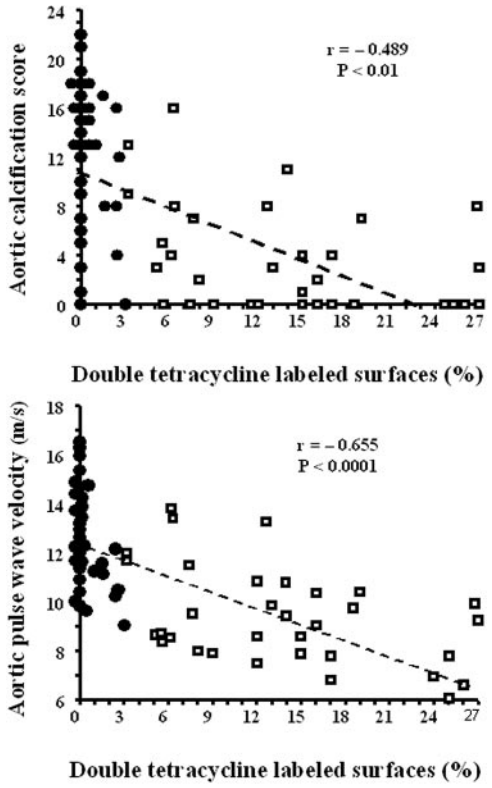

Figure 4. Correlations for the entire population (n = 66) between double tetracycline–labeled surfaces and abdominal aortic calcification score or aortic PWV. ●, adynamic bone; □, active bone.

sociated with Ca load and bone activity, with a significant Ca dosage*bone activity interaction. In multivariate analyses, the β coefficient of the interaction term was negative, indicating that the correlation between Ca load and PWV differed according to bone activity and that the presence of ABD conferred significantly greater influence of Ca load. Independent of any other factor, including dosage of Ca-containing PO_4 binders, adynamic bone status was associated with greater aortic stiffening (Figure 4).

We can only speculate about the mechanisms linking bone activity with aortic stiffening and arteriosclerosis. Bone remodeling is regulated by multiple hormones, including those involved in endocrine regulation of energy metabolism, such as leptin.[29] In chronic renal failure, serum leptin is increased and inversely correlated to histomorphometric parameters of bone turnover and PTH,[30,31] suggesting that leptin might be implicated in low bone turnover and AC.[32] In a recent study, Lee *et al.*[33] showed that osteoblasts exert an endocrine regulation on energy metabolism. Those authors showed that mice lacking the protein tyrosine phosphatase OST-PTP are hypoglycemic and protected from obesity and glucose intolerance associated with β cell proliferation and insulin secretion. All of these phenotypic characteristics could be corrected by removing one osteocalcin allele. Osteocalcin can improve glucose tolerance and stimulate insulin expression in β cells and adiponectin in adipocytes.[33] Adiponectin protects arteries against hypertension, suppresses atherosclerosis, and increases bone mass by activating osteoblastogenesis.[34]

The clinical relevance of bone–Ca interaction needs further research. Recent randomized study of hemodialysis patients did not demonstrate a higher mortality in patients who were treated with Ca-containing PO_4 binders[35] but indicated a significant age effect in patients who were older than 65 yr. Aging adversely affects bone formation. An association of Ca supplementation with upward trend in cardiovascular event rates was recently observed in elderly healthy postmenopausal women.[36]

Patients with ESRD and active bone were less sensitive to the use of Ca-containing PO_4 binders, and many patients had no AoC even after 15 yr on hemodialysis (Table 2). These latter were younger, did not have malnutrition inflammation, and took lower dosages of Ca-containing PO_4 binders. The practical clinical problem remains how to identify precisely patients who are more prone to aortic calcifications. Patients with active bone differ from those with ABD, because they have significantly higher PTH (and other biochemical markers), but as shown in Figure 3, serum PTH levels overlapped between the two groups, and its specificity and sensitivity to predict the degree of bone turnover has been questioned. It is excluded on ethical grounds to biopsy bone merely to evaluate the need for PO_4 control, and it is essential to design studies to identify and define the most specific and sensitive noninvasive markers of bone turnover in patients with ESRD.[37]

This study has several limitations. The first is the observational cross-sectional nature of the study and that prevalent patients were evaluated, thereby making it difficult to reconstruct the natural history of bone disease and its association with AoCS and stiffness. The second concerns some clinical characteristics of this population that were typical for patients treated in the Ile-de-France/Paris region in the late 1980s and in 1990s, before the introduction of non–Ca-containing PO_4 binders and calcimimetics.[38] Many included patients had been on hemodialysis for many years and had begun replacement therapy when some therapeutic approaches, no longer recommended today, were used, principally exposure to Al-containing PO_4 binders and total surgical PTX. Although Al-containing PO_4 binders are no longer recommended, they are still frequently used, and Al still seems to be implicated in a high percentage of low-turnover bone disease.[39,40] With the introduction of calcimimetics into the therapeutic armamentarium, the incidence of surgical PTX has decreased but not disappeared, and postsurgical hypoparathyroidism is still seen and represents a major cause of hypoparathyroidism.[41]

In conclusion, in hemodialysis patients with ESRD, aortic stiffness and calcifications were significantly associated with both Ca load and bone activity, with a significant Ca load*bone activity interaction. The positive associations between Ca load and PWV or AoCS were stronger in patients with ABD, indicating that the presence of ABD conferred significantly greater influence of Ca load on aortic calcifications and stiffening. Independent of any other factor, including dosage of Ca-con-

taining PO_4 binders, adynamic bone status was associated with greater aortic stiffening, suggesting a direct bone–arterial cross-talk.

CONCISE METHODS

Patients

Inclusion criteria were (*1*) hemodialysis vintage at least 12 mo (median 72; range 12 to 214); (*2*) age ≥18 and ≤70; (*3*) absence of clinical history of cardiovascular disease; and (*4*) complete set of results including blood chemistries, AoCS, bone biopsy, and aortic PWV. Between 1986 and 1996, 66 patients who had ESRD and fulfilled these criteria (48 patients were also part of a previous cohort[12]) were included. Dialysis duration was individually tailored (4 to 6 h thrice weekly) to control body fluids and blood chemistries and to achieve a Kt/V >1.2 (1.42 ± 0.11). Bicarbonate dialysate was prepared using double reverse osmosis-treated water with 1.25, 1.5, or 1.75 mmol/L of Ca, according to the serum Ca-PO_4 equilibrium and the need to use vitamin D_3 (1α-OH-D_3) and $CaCO_3$. Although $CaCO_3$ was used exclusively as a PO_4 binder at the time of the study, 28 patients had taken Al hydroxide [$Al(OH)_3$] in the past. Twelve patients underwent subtotal PTX and 10 patients total PTX with heterotopic autotransplantation into the forearm. PTX had been performed 20 to 70 mo before the study. Eight patients had post-PTX hypoparathyroid activity (PTH <100 pg/ml). Patients regularly took iron and vitamin supplements.

Abdominal Aortic Calcification Score

Lateral lumbar spine radiographs were acquired in the standing position, as described previously.[40] An AoCS was developed to grade AoC severity at the level of the first four (L1 through L4) lumbar vertebrae. Radiographs were read by two independent observers with no knowledge of the patients' clinical histories. The radiodensity of the aortic wall was systematically assessed at each vertebral segment, and calcific deposits were considered present when densities were visible in an area parallel to the lumbar spine and anterior to the lower part of the spine. Calcific densities were graded 0 to 3 at each lumbar vertebral segment: 0, no calcific deposits; 1, small scattered calcific deposits filling less than one third of the longitudinal aorta wall; 2, one third or more but less than two thirds of the longitudinal aorta wall calcification; and 3, two thirds or more of the longitudinal aorta wall calcification. A separate score was determined for the anterior and posterior aorta, and the values were summed across the four vertebral levels, yielding in an abdominal AoCS that could range from 0 to 24 points, as described previously.[5,42] It has been shown that the AoCS is an important predictor of vascular morbidity and mortality, and the results of one study demonstrated very good correlation between AoCS and coronary calcification scores using electron-beam computed tomography.[43]

Aortic PWV, as a surrogate of aortic stiffness, was determined using the foot-to-foot method, as described previously.[44] Simultaneously recorded pulse waveforms were obtained transcutaneously over the common carotid and femoral groin arteries. PWV was calculated as the distance between suprasternal notch and femoral artery recording site measured over the surface of the body, divided by the time interval between the feet of the flow waves. This interval was averaged over 10 cardiac cycles. Aortic PWV (and corresponding BP) was measured at monthly intervals twice before bone biopsy and twice after bone biopsy. Reported PWV and BP values are the average of these multiple measurements.

Bone Histomorphometry

Diagnostic anterior iliac crest bone biopsies were taken after double tetracycline labeling according to the schedule of 2 d on tetracycline, 10 d off, and 2 d on.[12,45] On three 5-μm-thick sections stained with Toluidine blue, trabecular bone volume (%), osteoid surface and volume (%), osteoblast surface (%), osteoclast resorption surface (%), and osteoclast number (n/mm^2) were assessed. On two unstained 10-μm-thick sections, the bone mineralization rate and the extent of double and total tetracycline-labeled surfaces (%) were evaluated. Bone Al-staining was evaluated according to the method described by Maloney *et al.*[46] and expressed as the percentage of the trabecular surface stained. All measurements were made using an eye-piece reticle (Zeiss integral plate II, Oberkochen, Germany). The double tetracycline–labeled surfaces were used to distinguish between patients with ABD and those with active bone. The median value of double tetracycline–labeled surfaces (3.1%; range 0 to 27) was used to classify patients with ABD <3.1% (n = 33) and those with active bone ≥3.1% (n = 33).

Blood Chemistries

Blood chemistries including serum Ca and PO_4, blood lipids, intact PTH (Nichols Institute; N-IRMA), serum albumin, and serum Al were determined the week preceding the bone biopsy. Serum Al was measured with atomic absorption spectrophotometry and graphite furnace. Plasma samples for quantification of the Al concentration were obtained before routine hemodialysis and 40 h after deferoxamine infusion (40 mg/kg) after hemodialysis. Smoking habits, prescriptions for 1α-OH-D_3 (μg/wk), and the $CaCO_3$ dosage expressed in grams of elemental Ca/d prescribed to each patient were obtained from the patients' files. The average blood chemistry values and mean daily $CaCO_3$ dosage over the 12 mo preceding bone biopsy are reported. All patients gave informed written consent to participate in the study, which was approved by our institutional review board.

Statistical Analysis

Data are expressed as means ± SEM or medians (95% confidence intervals) when appropriate. The primary analysis concerned patient subgroup comparison (*i.e.*, active bone *versus* ABD). The secondary analyses concerned comparisons of patients with positive *versus* negative AoCS. Between-group comparisons for quantitative variables were performed using the Mann-Whitney U test, and χ^2 test was used for categorical variables. Pearson or Spearman correlation coefficient was used to assess the relationships between AoCS or aortic PWV and clinical or biochemistry parameters. The impact of interactions between Ca load (elemental Ca in g/d) and bone activity (expressed as dummy variables [*i.e.*, ABD, 0; active bone, 1]) on AoCS or aortic PWV was tested using ANOVA adjusted for covariates. The $CaCO_3$

load*bone activity interaction term was included in the multivariate regression analysis with the subset of independent clinical and biochemistry variables ($P < 0.05$ after correction for the number of correlations studied) associated with AoCS or PWV. All tests were performed using NCSS 7.0 software (J. Hintze, Kaysville, UT).

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DISCLOSURES

None.

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