

Reduction of Dialysate Calcium Level Reduces Progression of Coronary Artery Calcification and Improves Low Bone Turnover in Patients on Hemodialysis

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ABSTRACT

Exposure to high Ca concentrations may influence the development of low-turnover bone disease and coronary artery calcification (CAC) in patients on hemodialysis (HD). In this randomized, controlled study, we investigated the effects of lowering dialysate Ca level on progression of CAC and histologic bone abnormalities in patients on HD. Patients on HD with intact parathyroid hormone levels ≤ 300 pg/ml receiving dialysate containing 1.75 or 1.50 mmol/L Ca ($n=425$) were randomized to the 1.25-mmol/L Ca (1.25 Ca; $n=212$) or the 1.75-mmol/L Ca (1.75 Ca; $n=213$) dialysate arm. Primary outcome was a change in CAC score measured by multislice computerized tomography; main secondary outcome was a change in bone histomorphometric parameters determined by analysis of bone biopsy specimens. CAC scores increased from 452 ± 869 (mean $\pm$ SD) in the 1.25 Ca group and 500 ± 909 in the 1.75 Ca group ($P=0.68$) at baseline to 616 ± 1086 and 803 ± 1412 , respectively, at 24 months ($P=0.25$). Progression rate was significantly lower in the 1.25 Ca group than in the 1.75 Ca group ($P=0.03$). The prevalence of histologically diagnosed low bone turnover decreased from 85.0% to 41.8% in the 1.25 Ca group ($P=0.001$) and did not change in the 1.75 Ca group. At 24 months, bone formation rate, trabecular thickness, and bone volume were higher in the 1.25 Ca group than in the 1.75 Ca group. Thus, lowering dialysate Ca levels slowed the progression of CAC and improved bone turnover in patients on HD with baseline intact parathyroid hormone levels ≤ 300 pg/ml.

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Vascular calcifications (VCs), which occur more frequently and progress more rapidly in patients on hemodialysis (HD)^{1,2} compared with the general population, are associated with a high rate of cardiovascular events.^{3–5} Medial VC, frequently encountered in uremic patients, is associated with arterial stiffness, left ventricular hypertrophy and altered coronary perfusion, heart failure, and poor survival.^{6,7} In addition to the traditional risk factors, disturbances of mineral metabolism are probably among the key risk factors in the development and progression of VC in patients on HD.⁸ These patients may have positive calcium (Ca) balance secondary to use of Ca-based phosphate

binders, high doses of vitamin D analogs, and high dialysate Ca concentrations. In turn, high Ca load is associated with more VC.^{9–11}

Low-turnover bone disease has been found to be a frequent type of renal osteodystrophy in

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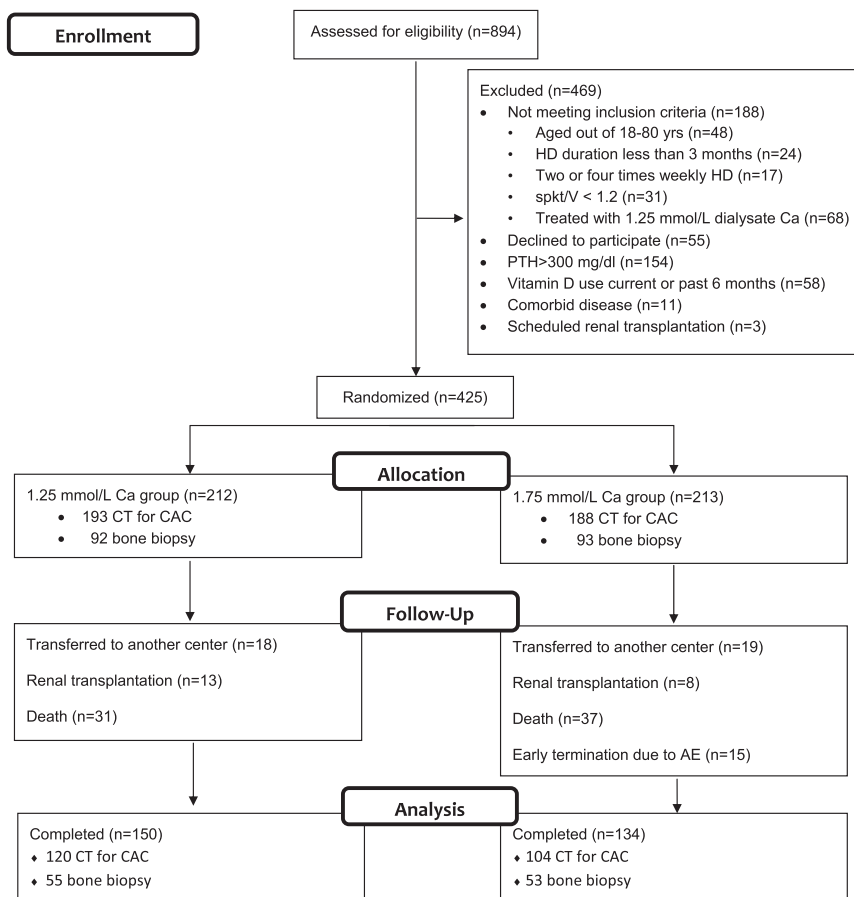


Figure 1. Disposition of patients. Among 894 patients treated in eight dialysis clinics, 425 subjects were randomized to either 1.25 or 1.75 mmol/L dialysate Ca groups. After 24 months of follow-up period, 284 patients completed the study (150 in the 1.25 and 134 in the 1.75 Ca groups). AE, adverse event; CT, computerized tomography; spKt/V, single pool Kt/V.

patients on dialysis.¹² Positive Ca balance may lead to over-suppression of parathyroid gland and thus, contribute to the pathogenesis of low-turnover bone disease¹³ with the associated VC.¹⁴ Low-turnover bone disease is associated with low capacity of Ca incorporation into bone and an inability to buffer extra Ca load,¹⁵ which could explain the association between low-turnover bone disease and VC.^{14,16–18}

The effects of lowering Ca load on bone disease and coronary artery calcification (CAC) in patients on HD by the use of non-Ca-based phosphate binders have been evaluated in numerous studies.^{3,10,19} However, the effect of lowering Ca exposure through dialysate²⁰ on CAC and renal osteodystrophy is largely unknown. Available recommendations²¹ regarding use of dialysate Ca are not validated by studies evaluating effects on CAC and bone.

This study tests the hypothesis that lowering Ca exposure through dialysate reduces progression of CAC and increases bone turnover in patients on HD with baseline parathyroid hormone (PTH) levels ≤ 300 pg/ml treated with 1.75 or 1.50 mmol/L dialysate Ca.

RESULTS

Patient Selection—Study Flow

All 894 patients treated in eight dialysis clinics were assessed for eligibility (Figure 1). In total, 469 patients were excluded because of the following reasons: (1) not meeting inclusion criteria ($n=188$), (2) declined to participate ($n=55$), (3) PTH values >300 pg/ml ($n=154$), (4) vitamin D use current or during 6 months before randomization ($n=58$), (5) expecting living donor renal transplantation ($n=3$), or (6) serious life-limiting comorbid conditions, including active malignancy, chronic infection, and end stage cardiac, pulmonary, or hepatic disease ($n=11$). In total, 425 subjects met the selection criteria and were assigned in a 1:1 ratio to either 1.25- or 1.75-mmol/L dialysate Ca groups (1.25 and 1.75 Ca groups).

During 24 months of follow-up, 68 patients died (28 from cardiovascular causes). Overall mortality was not different between the groups (7.86 and 9.84 per 100 patient-years in the 1.25 and 1.75 Ca groups, respectively; $P=0.29$); 21 patients (13 in the 1.25 and 8 in the 1.75 Ca groups) had renal transplantation, and 37 (18 in the 1.25 and 19 in the 1.75 Ca groups) moved to nonparticipating centers. In the 1.75 Ca group, the study was terminated prematurely in 15 patients (7%) because of hypercalcemia. No patient dropped out of the study because of hyper- or hypocalcemia in the 1.25 Ca group; 284 patients completed the study (150 in the 1.25 and 134 in the 1.75 Ca groups).

Baseline Characteristics

Patients in the two groups were matched with respect to baseline characteristics and concomitant therapy (Table 1). At baseline, 11.8% of the patients in the 1.25 Ca group and 12.7% of the patients in the 1.75 Ca group had hyperphosphatemia (>5.5 mg/dl; $P=0.84$). There was no difference between the 1.25 and 1.75 Ca groups in the percentage of patients with PTH levels <150 pg/ml (84.2% and 82.9%, respectively; $P=0.78$). Daily urine volume was <250 ml in $>95\%$ of the patients, and none of them were treated with diuretics.

Changes in Clinical, Biochemical, and Medication Data

Time-averaged clinical and biochemical parameters and medication data are shown in Table 2. There was no difference in BP and the frequency of intradialytic hypotension episodes between the groups. Two bone fractures occurred during 2 years (one in each group). Biochemical results showed that

Table 1. Baseline patient characteristics and medication data (mean±SD)

Variables	1.25 Ca Group (n=212)	1.75 Ca Group (n=213)	P Value
Age, yr	59.3±13.8	59.9±13.5	0.63
Women, %	41	46	0.30
Diabetes, %	24.0	27.2	0.45
Time on HD, mo	51.2±38.9	50.5±44.1	0.86
Cardiovascular disease in history, %	21.1	21.9	0.85
Smoking, %	28	30	0.78
Causes of ESRD, %			
Diabetic nephropathy	24.0	27.2	
Glomerulonephritis	8.8	6.6	
Hypertension	13.8	14.2	
Other	33.3	36.4	
Body mass index, kg/m ²	24.1±4.0	24.2±4.2	0.78
Interdialytic weight gain, kg	2.2±0.9	2.3±0.9	0.26
Hypotension episodes, n/1000 sessions	63.4	64.1	0.84
Systolic BP, mmHg	124±15	126±16	0.25
Diastolic BP, mmHg	74±9	75±9	0.18
Biochemical parameters			
Predialysis urea, mg/dl	133±28	134±25	0.52
Predialysis creatinine, mg/dl	8.5±1.8	8.3±1.8	0.14
Potassium, mEq/L	4.9±0.6	4.9±0.6	0.85
Ca, mg/dl	9.18±0.63	9.12±0.62	0.29
P, mg/dl	4.31±1.02	4.40±0.97	0.31
Ca-P product, mg ² /dl ²	39.6±9.8	40.1±9.1	0.60
Alkaline phosphatase, U/L	104±53	103±59	0.81
PTH, pg/ml	84±71	86±71	0.72
Equilibrated Kt/V	1.42±0.23	1.44±0.23	0.78
Albumin, g/dl	3.91±0.27	3.92±0.26	0.72
Total cholesterol, mg/dl	182±44	181±42	0.83
Triglyceride, mg/dl	165±98	164±113	0.88
HDL-cholesterol, mg/dl	40.9±11.5	41.7±12.0	0.66
LDL-cholesterol, mg/dl	108±35	107±34	0.94
Hemoglobin, g/dl	11.0±1.1	11.0±1.3	0.59
Bicarbonate, mEq/L	24.5±3.6	23.9±3.3	0.28
C-reactive protein, mg/dl	1.32±1.95	1.52±2.14	0.83
Dialysate Mg, mmol/L	1.0	1.0	NA
Dialysate Ca before the study, %			0.78
1.75 mmol/L	64.4	63.5	
1.50 mmol/L	35.6	36.5	
Use of P binder, %	58	60	0.73
Dose of P binder (elementary Ca), mg/d	535±685	598±797	0.38
CAC score, ^a Agatston	587±1369	551±967	0.77
CAC score, ^a volume	466±1084	444±755	0.82
Bone formation rate, ^b mm ³ /cm ² per yr	1.14±1.39	1.25±1.50	0.60
Bone volume/tissue volume, ^b %	18.2±7.7	19.3±6.3	0.31
Mineralization lag time, ^b d	156±196	137±213	0.61
Low bone turnover, ^b %	79.6	78.3	0.82

P, phosphorus; Mg, magnesium; NA, not applicable; P binder, phosphate binder.

^an=193 in the 1.25 Ca group and n=188 in the 1.75 Ca group.^bn=93 in the 1.25 Ca group and n=92 in the 1.75 Ca group.

serum Ca levels were lower and that serum phosphorus, PTH, and alkaline phosphatase levels were higher in the 1.25 Ca group than in the 1.75 Ca group; episodes of hypercalcemia were more frequent in the 1.75 Ca group than in the 1.25 Ca group (11.3 versus 4.4 per 100 patient-months, respectively; $P<0.001$). The mean dose of elemental Ca

given as a phosphate binder was similar in the two groups as well as the frequency of phosphate binder use (Supplemental Table 1). In the 1.25 Ca group, more patients received intravenous calcitriol compared with those in the 1.75 Ca group (38.7% and 13.0%, respectively; $P<0.001$); calcitriol use was more frequent in the 1.25 Ca group at all

Table 2. Time-averaged data during the 24-month study^a (mean±SD)

Variables	1.25 Ca Group (n=212)	1.75 Ca Group (n=213)	P Value
Clinical parameters			
Body mass index, kg/m ²	23.7±3.9	24.0±4.38	0.26
Post-HD body weight, kg	65.5±11.9	65.3±12.6	0.85
Systolic BP, mmHg	122.7±13.2	123.7±12.0	0.38
Diastolic BP, mmHg	74.7±7.1	75.0±6.2	0.54
Hypotension episodes, n/1000 sessions	64.4	69.1	0.66
Biochemical parameters			
Equilibrated Kt/V	1.45±0.21	1.45±0.23	0.83
Albumin, g/dl	4.01±0.28	3.98±0.27	0.25
Ca, mg/dl	8.96±0.57	9.40±0.61	<0.001
P, mg/dl	4.92±1.03	4.58±1.00	0.001
Ca-P product, mg ² /dl ²	44.1±9.8	43.1±10.5	0.32
Alkaline phosphatase, U/L	139±72	101±52	<0.001
PTH, pg/ml	297±228	141±120	<0.001
Bicarbonate, mmol/L	22.2±1.5	21.7±1.7	0.001
Total cholesterol, mg/dl	161±39	164±41	0.45
Triglyceride, mg/dl	160±85	177±121	0.09
HDL-cholesterol, mg/dl	42.7±10.4	43.2±10.4	0.63
LDL-cholesterol, mg/dl	86.7±28.3	85.9±30.3	0.78
C-reactive protein, mg/dl	1.71±2.30	1.65±1.95	0.77
Medication data			
Use of phosphate binder at 24 mo, %	58	52	0.30
Dose of time-averaged elemental Ca, mg/d	695±496	663±546	0.53
Use of iv calcitriol vitamin D at 24 mo, %	38.7	13	<0.001
Dose of time-averaged vitamin D, µg/wk	0.30±0.46	0.09±0.30	<0.001
Mean erythropoietin dose, U/wk	2373±2403	2789±2313	0.07
Use of antihypertensive drug at 24 mo, %	14.5	16.4	0.76

P, phosphorus.

^aExcept frequencies of medication use at 24 months (n=150 in the 1.25 Ca group and n=134 in the 1.75 Ca group).

time points starting from the 12th month (Supplemental Table 1).

Changes in CAC Scores

CAC scores were measured in 89% of the patients at baseline. Baseline characteristics were not different between patients who underwent CAC score measurement (n=381) and those who did not (n=44; 19 patients unable to attend appointment, 13 withdrew consent, and 12 had cardiac arrhythmia) (Supplemental Table 2).

Of 381 patients who had initial CAC score measurement, 262 completed follow-up (death, 54; transfer to nonparticipating centers, 32; transplantation, 18; and dropout, 15). The second CAC score measurement could not be done in 38 of these 262 patients (11 patients unable to attend appointment, 26 withdrew consent for second measurement, and one had cardiac arrhythmia). Baseline characteristics were similar between patients who had two CAC score measurements (n=224) and those who only had the first CAC score measurement (n=157); only PTH and hemoglobin were different between these two groups (Supplemental Table 2). Baseline CAC score tended to be slightly higher in patients without final CAC measurement (P=0.08). Death was the major reason (34.4%) for not having a second CAC measurement. The 54 patients who died during the study had significantly higher

mean baseline CAC scores compared with survivors (n=327; 1040±2067 and 491±951, respectively; P=0.02).

Progression of CAC score was evaluated in 224 patients who had two CAC score measurements (120 in the 1.25 Ca group and 104 in the 1.75 Ca group). There was no difference between the 1.25 and 1.75 Ca groups regarding baseline characteristics (Supplemental Table 3). Mean CAC scores were also similar between treatment groups at baseline (452±869 in the 1.25 Ca group and 500±909 in the 1.75 Ca group; P=0.68) (Table 3). Also, the frequency of patients with CAC score <100 was similar in the 1.25 and 1.75 Ca groups (53.3% and 47.1%, respectively; P=0.85). CAC score increased in both groups within 2 years (Table 3). Although CAC score at 24 months was lower in the 1.25 Ca group than in 1.75 Ca group, the difference was not statistically significant (616±1086 and 803±1412, respectively; P=0.25). However, the progression rate of CAC scores, which was the primary outcome measure, was significantly less in the 1.25 Ca group than in the 1.75 Ca group (160±299 versus 303±624, respectively) (Table 4). The mean absolute difference in progression of CAC scores between the 1.25 and 1.75 Ca groups assessed by Agatston method was −138 (P=0.03), and the mean absolute difference in progression of volume scores was −118 (P=0.01). The difference between changes in square root-transformed CAC

Table 3. CAC scores in treatment groups at baseline and at the end of study

CAC score	1.25 Calcium Group (n=120)			1.75 Calcium Group (n=104)		
	Baseline	Month 24	P Value	Baseline	Month 24	P Value
CAC score—Agatston, mean±SD	452±869	616±1086	<0.001	500±909	803±1412	<0.001
CAC score—Agatston, median (interquartile range)	63 (0–504)	99 (0–661)	<0.001	135 (0–586)	258 (10–945)	<0.001
CAC score—volume, mean±SD	351±679	466±821	<0.001	383±698	617±1089	<0.001
CAC score—volume, median (interquartile range)	46 (0–364)	67 (0–530)	<0.001	116 (0–426)	199 (8–728)	<0.001

Table 4. Mean difference in changes of CAC scores between the groups (mean±SD)

Changes in CAC Scores	1.25 Calcium Group (n=120)	1.75 Calcium Group (n=104)	Mean Difference between Groups (95% Confidence Interval)	P Value
Absolute difference				
ΔCAC score—Agatston	160±299	303±624	−138 (−265 to −12)	0.03
ΔCAC score—volume	115±208	234±482	−118 (−214 to −22)	0.01
Transformed difference				
ΔCAC score—Agatston	3.01±3.94	4.79±6.22	−1.77 (−3.13 to −0.42)	0.01
ΔCAC score—volume	2.50±3.38	4.21±5.46	−1.70 (−2.88 to −0.52)	<0.01

volume scores in the two groups was also significant ($P<0.01$). Adjustment for baseline CAC score did not change the results.

Univariate analysis revealed a positive association between progression of CAC score and baseline CAC score ($\text{Rho}=0.507$; $P<0.001$), age ($\text{Rho}=0.194$; $P=0.003$), and time-averaged levels of serum Ca ($\text{Rho}=0.202$; $P=0.002$) and phosphorus ($\text{Rho}=0.133$; $P=0.04$). There was an inverse correlation between CAC progression and alkaline phosphatase levels ($\text{Rho}=-0.289$; $P<0.001$). We found no association between the dose of Ca-based phosphate binder and CAC progression ($P=0.52$). In stepwise regression analysis for progression of CAC adjusted for demographics, medications, and biochemical data, the following factors were found to be significant predictors: baseline CAC score, Ca-phosphorus product, age, and dialysate Ca group ($r^2=0.43$; $P<0.001$).

In *post hoc* analysis, we observed a significant interaction between time-averaged serum phosphorus level and treatment arm on the progression of CAC score outcome by the Agatston score (P interaction = 0.001). We, therefore, dichotomized participants at the mean time-averaged phosphorus level (<4.7 mg/dl versus ≥ 4.7 mg/dl) and evaluated the effect of randomization in each strata. We observed that randomization to 1.75 mmol/L Ca concentration dialysate was associated with higher CAC progression rates in patients with time-averaged phosphate ≥ 4.7 mg/dl than in those randomized to 1.25 mmol/L Ca arm (Δ Agatston CAC scores of 171 ± 324 in the 1.25 Ca group and 485 ± 801 in the 1.75 Ca group; $P<0.01$), whereas progression rates were similar irrespective of dialysate Ca bath in those with lower time-averaged serum phosphate levels (Δ Agatston CAC score of 157 ± 274 versus 158 ± 386 , respectively; $P=0.98$) (Figure 2).

Changes in Bone Histomorphometric Parameters

At baseline, bone biopsy was performed in 185 patients. Baseline characteristics were not different between patients who underwent bone biopsy ($n=185$) and those who did not ($n=240$) (Supplemental Table 4).

Of 185 patients who had a baseline biopsy, 137 completed the follow-up (death in 20, transplantation in 11, transfer to nonparticipating centers in 14, and dropout in three). Second bone biopsies were done in 108 of 137 patients, because 29 patients did not agree to a second biopsy.

Baseline characteristics were similar between patients who had two biopsies ($n=108$) and those who had only a first biopsy ($n=77$) (Supplemental Table 4).

Bone turnover, mineralization, and bone volume parameters were similar at the baseline in the two groups (Table 5). Other characteristics were also not different at the baseline between the groups, except for lower frequency of cardiovascular disease history in the 1.25 Ca group (Supplemental Table 5).

Bone Turnover

Bone turnover is defined histomorphometrically by bone formation rate and activation frequency. Both of these parameters increased significantly in the 1.25 Ca group from baseline to month 24, whereas no changes were observed in the 1.75 Ca group (Table 5). The prevalence of histologically diagnosed low bone turnover decreased from 85.0% to 41.8% in the 1.25 Ca group ($P=0.001$) and did not change in the 1.75 Ca group (from 79.2% to 64.2%). There was a commensurate significant increase in the number of osteoblasts and osteoclasts in the 1.25 Ca group. In the 1.75 Ca group, the number of osteoclasts increased, with no change in osteoblast number.

Mineralization

Normal mineralization is defined by osteoid thickness $<20\ \mu\text{m}$ and mineralization lag time <100 days. Although mineralization lag times were long in both groups, osteoid thickness was normal (Table 5). Thus, overt osteomalacia was not found in any patients at baseline or the end of the study. No stainable bone aluminum was found at baseline or after 2 years of treatment. Osteoid thickness increased in the 1.25 Ca group but not to values

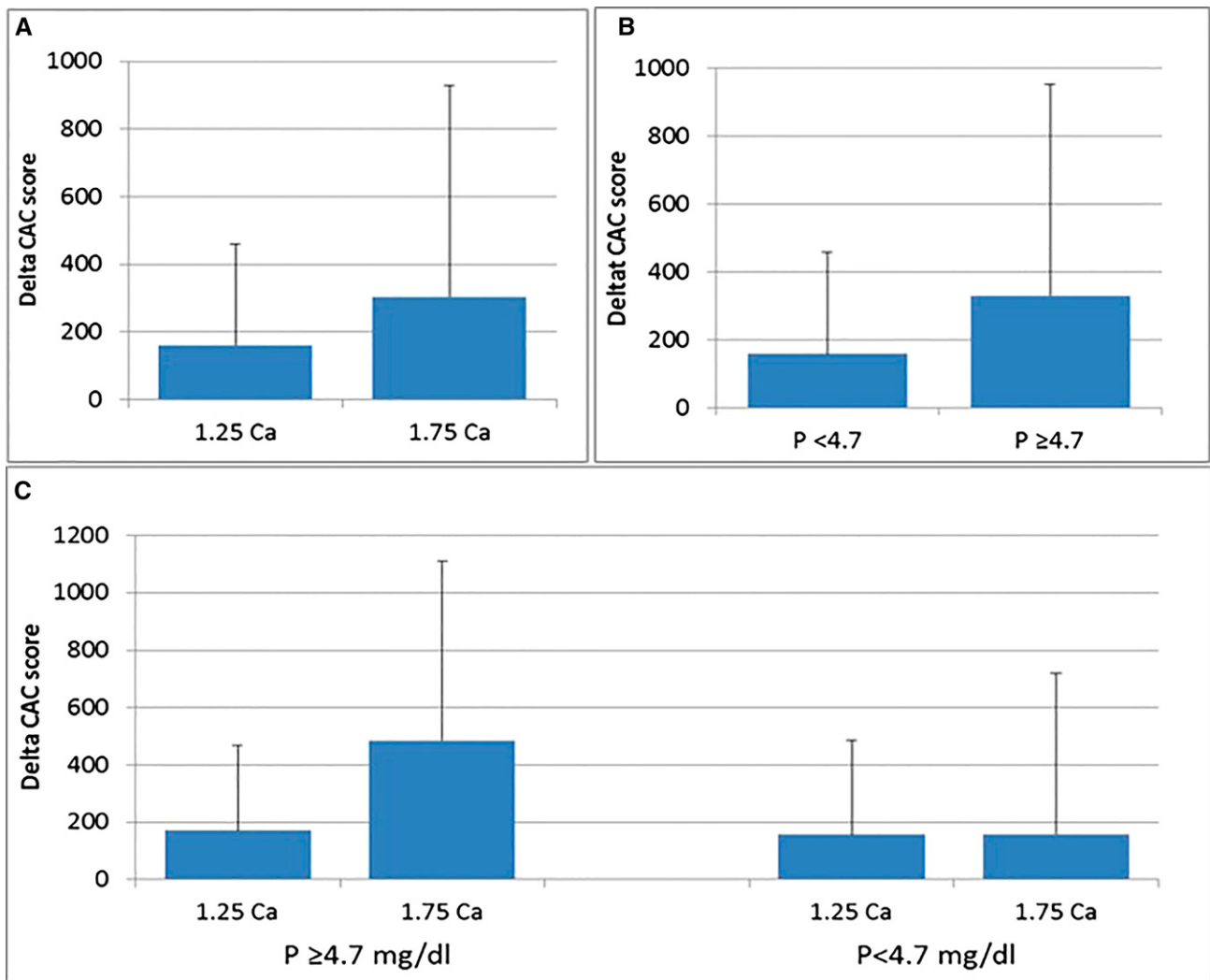


Figure 2. Higher CAC progression in the 1.75 Ca group than 1.25 Ca group (160 ± 299 versus 303 ± 624 , $P=0.03$) (A); in the whole group, higher CAC progression in patients with serum phosphorus level ≥ 4.7 compared to <4.7 mg/dl (292 ± 576 versus 160 ± 342 , $P=0.03$) (B); in patients with serum phosphorus level ≥ 4.7 mg/dl, higher CAC progression with 1.75 than 1.25 mmol/L dialysate Ca (485 ± 801 versus 171 ± 324 , $P=0.01$) (C); in patients with serum phosphorus level <4.7 mg/dl, similar CAC progression in the 1.75 and 1.25 Ca groups (157 ± 274 versus 158 ± 386 , $P=0.98$) (C).

above the normal range reflecting increased bone formation. In the 1.75 Ca group, there were no changes in osteoid thickness. Mineralization lag time did not change significantly in either group, whereas statistically but not physiologically relevant decreases (within the normal range) were observed for osteoid maturation time in both groups. Lack of abnormally prolonged mineralization lag time combined with no increase in osteoid maturation time and osteoid thickness document absence of osteomalacia.

Volume

Mean cancellous bone volume was within the normal range (16.8%–22.9% cancellous bone per total bone volume) at baseline and 24 months in the 1.25 and 1.75 Ca groups but increased significantly from low-normal to high-normal in the 1.25 Ca group but not the 1.75 Ca group (Table 5).

Similarly, trabecular thickness increased in the 1.25 Ca group but not in the 1.75 Ca group. Cortical thickness did not change in either group, but cortical porosity increased in both groups.

Adherence to the Treatment and Adverse Events

All patients in the 1.25 Ca group received randomized treatment during follow-up. In the 1.75 Ca group, the study was terminated prematurely in 7% of the patients because of hypercalcemia. There were no cross-overs during follow-up. Adverse events occurred in 95 (44.8%) patients in the 1.25 Ca group and 117 (54.9%) patients in the 1.75 Ca group ($P=0.04$). Serious adverse events were reported in 79 (37.2%) patients in the 1.25 Ca group and 97 (45.5%) patients in the 1.75 Ca group ($P=0.08$) (Table 6).

Table 5. Bone histomorphometric parameters in treatment groups at baseline and the end of study (mean±SD)

Bone Histomorphometry Parameters	1.25 Calcium Group (n=55)			1.75 Calcium Group (n=53)		
	Baseline	Month 24	P Value	Baseline	Month 24	P Value
Bone turnover						
Bone formation rate/bone surface (normal: 1.80–3.80 mm ³ /cm ² per yr)	1.03±1.33	3.24±2.77 ^a	<0.001	1.41±1.61	1.93±1.37	0.07
Activation frequency (normal: 0.49–0.72 yr ⁻¹)	0.22±0.28	0.67±0.56 ^a	<0.001	0.29±0.34	0.41±0.28	0.06
Osteoblast no./bone perimeter (normal: 10–200/100 mm)	61.5±87.2	144±199 ^b	<0.01	103±180	66.7±124	0.12
Osteoclast no./bone perimeter (normal: 1–53/100 mm)	28.9±31.2	48.0±44.8	<0.01	23.9±23.8	39.8±42.4	0.01
Mineralization						
Osteoid thickness (normal: <20 μm)	10.1±2.9	13.1±5.5	<0.001	11.1±4.8	11.0±6.1	0.87
Mineralization lag time (normal: <100 d)	165±178	126±127	0.09	109±95	83±73	0.17
Osteoid maturation time (normal: <35 d)	17.88±6.10	17.25±7.35	<0.001	16.12±6.33	15.21±8.39	<0.001
Volume						
Bone volume/tissue volume (normal: 16.8%–22.9%)	18.6±8.0	21.3±6.6 ^b	0.02	18.9±6.1	18.0±7.1	0.43
Trabecular thickness (normal: 99–142 μm)	90.6±28.7	98.5±24.7 ^c	0.05	93.1±23.3	88.4±25.9	0.26
Cortical thickness (normal: 0.52–1.65 μm)	710±530	550±350	0.14	620±470	540±560	0.33
Cortical porosity (normal: 1.9%–10%)	2.47±2.22	14.78±7.34	<0.001	2.69±2.75	10.88±6.27	<0.001

^aP=0.03 (P value of the difference between 1.25 and 1.75 Ca groups at the end of the study).^bP=0.01 (P value of the difference between 1.25 and 1.75 Ca groups at the end of the study).^cP=0.04 (P value of the difference between 1.25 and 1.75 Ca groups at the end of the study).

DISCUSSION

This randomized clinical trial shows that lowering dialysate Ca levels slows down progression of CAC and improves bone turnover in patients on HD with baseline PTH levels ≤300 pg/ml while under treatment with ≥1.50 mmol/L Ca dialysate.

Although the CAC score in the 1.25 Ca group was not significantly different from that in the 1.75 Ca group at the end of the study, the progression rate of CAC was markedly lower in the 1.25 Ca group compared with the 1.75 Ca group. This confirms a role of high dialysate Ca exposure in the progression of CAC.

Table 6. Monitored adverse events during the study period between the groups

Adverse Events	1.25 Ca Group (n=212) No. of Patients (%)	1.75 Ca Group (n=213) No. of Patients (%)	P Value
Any adverse event	95 (44.8)	117 (54.9)	0.04
Any serious adverse event	79 (37.2)	97 (45.5)	0.08
Requiring permanent discontinuation ^a	0	15 (7.0)	NA
Death	31 (14.6)	37 (17.3)	0.44
Cardiovascular causes	13 (6.1)	15 (7.0)	0.70
Sudden death	6 (2.8)	4 (1.8)	0.51
Noncardiovascular causes	18 (8.4)	22 (10.3)	0.51
Infection ^b	7 (3.3)	10 (4.6)	0.46
Other ^c	11 (5.1)	12 (5.6)	0.83
Nonfatal cardiovascular events ^d	3 (1.4)	6 (2.8)	0.31
Infection ^e	9 (4.2)	11 (5.1)	0.65
Gastrointestinal disorders ^f	6 (2.8)	3 (1.4)	0.35
Hepatic disorders ^g	2 (0.9)	0	NA
Hospitalization ^h	27 (12.7)	24 (11.2)	0.64
Fracture	1 (0.4)	1 (0.4)	>0.99

NA, not applicable.

^aOnly reason for permanent discontinuation was hypercalcemia in the 1.75 Ca group.^bPneumonia, diabetic foot infections, and catheter infections were the most frequent reasons.^cMalignancy, pulmonary embolism, and gastrointestinal hemorrhage were most frequently reported.^dStroke, unstable angina pectoris requiring hospitalization, and acute myocardial infarction.^ePneumonia and diabetic foot infection were most frequently reported.^fGastrointestinal hemorrhage was the most frequent reason.^gTwo acute cholecystitis episodes.^hVascular access problems and infections were most frequently reported.ⁱFemoral neck fracture in each group.

As a result of treatment with Ca-based phosphate binders, vitamin D, and 1.75 or 1.50 mmol/L Ca dialysate, many patients on HD may be in positive Ca balance. This may contribute to development of low bone turnover and CAC.^{9,22} Use of non-Ca-based phosphate binders to reduce Ca exposure has been claimed to stop progression of CAC in patients on prevalent HD and patients on incident HD.^{2,10} However, this was not confirmed in another study reporting equal progression rates in non-Ca- and Ca-based phosphate binders groups when similar LDL cholesterol levels were achieved with the use of statins.³ The effect of non-Ca-based phosphate binders on bone histomorphometry has been investigated only in small studies with inconclusive results.^{19,23–25}

The effect of dialysate Ca, an important source of Ca exposure, on VC and bone disease in patients on HD has not been investigated before in a randomized, controlled trial. Dialysate Ca levels of 1.50–1.75 mmol/L may result in transfer of Ca and thus, increasing serum Ca levels. A mean accumulation of 879 mg Ca per HD session was found when patients with a predialysis serum Ca level of 2.27 mmol/L (9.1 mg/dl) were dialyzed with a dialysate containing 1.75 mmol/L Ca.²⁰ In the same study, dialysate containing 1.25 mmol/L Ca did not cause net Ca flux, although another study reported a negative Ca balance with 1.25 mmol/L dialysate Ca.²⁶ Because serum Ca affects PTH secretion, Ca transfer from the dialysate containing 1.75 or 1.50 mmol/L Ca can be expected to suppress PTH levels and may result in lowering of bone turnover. Although low-turnover bone disease is often asymptomatic, it may increase bone fracture incidence, cause poor healing of fractures, and predispose to extraosseous calcification because of diminished buffering capacity of bone.^{15,16,27}

In the 1960s, when HD became available, a dialysate Ca concentration of 1.25 mmol/L was chosen to match physiologic blood Ca levels. In the era when vitamin D was not available and phosphate binders were aluminum based, the use of 1.25 mmol/L dialysate Ca resulted in an elevation of serum PTH levels. Therefore, dialysate Ca concentration was increased to 1.75 mmol/L. After the introduction of vitamin D treatment and replacement of aluminum-containing binders with Ca-based phosphate binders, dialysate Ca concentration was reduced back to 1.25 mmol/L, especially in the United States. In 2003, Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines recommended use of a 1.25 mmol/L dialysate Ca concentration on the basis of clinical experience rather than outcome data.²¹ Because of the lack of randomized studies proving superiority and safety of 1.25 mmol/L dialysate Ca concentration, the use of 1.50 or even 1.75 mmol/L dialysate Ca concentrations continued in many countries. In the early 2000s, the frequency of ≤ 1.25 mmol/L dialysate Ca use was 64.1% in the United States, 23.9% in Europe, and 19.8% in Japan.²⁸ In 2009, Kidney Disease Improving Global Outcomes guidelines advised a dialysate Ca concentration of 1.25 or 1.50 mmol/L, but again, this was not evidence based.²⁹

This randomized clinical trial produced evidence to favor reduction of dialysate Ca level from 1.75 or 1.50 mmol/L to

1.25 mmol/L in patients on HD with PTH levels ≤ 300 pg/ml. Reduced Ca exposure through dialysate was associated with less progression of CAC and improved bone turnover without a decrease in bone volume within 2 years. Although this study was not designed to evaluate hard end points, such as death rate and incidence of bone fracture, CAC, the primary outcome measure of this study, is a well known independent risk factor for mortality.⁶ Also, low-turnover bone disease, the secondary outcome measure, is known to be associated with abnormal Ca homeostasis¹⁵ and increased incidence of bone fractures.²⁷

This study showed significantly lower serum Ca levels and lower frequency of hypercalcemic episodes in the 1.25 Ca group compared with the 1.75 Ca group. PTH and alkaline phosphatase levels were higher in the 1.25 Ca group. Higher phosphorus levels in the 1.25 Ca group may be related to increased release of phosphorus from bone because of higher turnover and/or increased intestinal phosphorus absorption resulting from more vitamin D use³⁰; however, compliance with phosphate binders may be an additional factor affecting serum phosphate levels. Along with a rise in PTH levels, all bone turnover parameters dramatically increased in the 1.25 Ca group; thus, prevalence of low-turnover bone disease decreased from 85% to 41.8%. We do not have a clear explanation for the increase in osteoclasts and the slight rise in PTH observed in the 1.75 Ca group, which was not accompanied by changes in alkaline phosphatase levels. It can be speculated that there might be an inappropriate overuse of vitamin D before initiation that is screening in the study. The long mineralization lag times observed with normal osteoid maturation time and normal osteoid thickness in all study groups and the absence of changes in these parameters indicate that there was no osteomalacia present at baseline or the end of the study in all patients. This prolongation of mineralization lag time reflects low bone formation. The differences in osteoid maturation time between baseline and the end of the study are statistically significant, but they are so small that they are unlikely to be biologically relevant. In the 1.25 Ca group, we documented increases in cancellous bone volume and trabecular thickness. Higher cancellous bone volume has been reported to be associated with lower CAC scores.³¹ The rise in cortical porosity in both groups can be explained by higher concentrations of serum PTH. The effects of PTH levels on cancellous and cortical bone are well established in the literature.^{32–34} This study confirms these observations in a prospective manner.

Haris *et al.*³⁵ investigated the effect of lowering dialysate Ca levels from 1.62 to 1.00 mmol/L during 16 months in 51 patients with adynamic bone disease on peritoneal dialysis (24 patients in the 1.0 mmol/L dialysate Ca groups and 27 patients in 1.62 mmol/L dialysate Ca group). The results of this partially randomized small trial (only 14 patients on low Ca completed the study) clearly showed improved bone turnover, increased PTH levels, and fewer hypercalcemic episodes. This randomized, controlled study confirms these results but differs, because it was

performed in patients on HD, was of longer duration, and investigated not only bone histomorphometry but also, VC.

Considering significant interaction between serum phosphorus and dialysate Ca level on progression of CAC score in covariance analysis, additional *post hoc* analyses revealed that high Ca exposure increases progression of CAC mainly in the presence of hyperphosphatemia. In patients with time-averaged phosphorus levels <4.7 mg/dl, progression of CAC was much lower compared with that in those patients with phosphorus levels ≥ 4.7 mg/dl. More importantly, progression of CAC was not different in the 1.25 and 1.75 Ca groups in the absence of hyperphosphatemia. This indicates a key role of phosphate in CAC as shown in *in vitro* studies.³⁶ However, it should be noted that this is a *post hoc* analysis, and it does not prove that higher Ca exposure is safe in the absence of hyperphosphatemia in terms of CAC progression.

It has been described that the corrected QT interval is prolonged by use of 1.25 mmol/L dialysate Ca concentration and that dialysate Ca level <1.25 mmol/L is associated with increased risk of sudden death.^{37,38} Although the study was underpowered to detect differences in death outcomes, we found no difference between the groups regarding death, sudden death, cardiovascular death, and nonfatal cardiovascular events. Adverse events were more frequent ($P=0.04$), and serious adverse events tended to be higher ($P=0.08$) in the 1.75 Ca group, mainly because of hypercalcemia requiring permanent discontinuation.

Dialysate Ca of 1.25 mmol/L is used worldwide without supporting evidence. The strength of the study is the randomized, controlled, prospective approach to explore the effects of lowering the dialysate Ca level from 1.75 or 1.50 mmol/L to 1.25 mmol/L on VC and bone disease in patients with PTH levels ≤ 300 pg/ml.

This study has some limitations. First, the statistical power of the study was lower than hypothesized because of higher dropout rates and the significant increase in CAC score also observed in the 1.25 group, whereas no progression was assumed. Therefore, the difference between CAC scores between the two groups did not reach statistical significance at the end of the study. However, progression rate of CAC score was significantly lower in the 1.25 Ca group. Second, in this study, reduction of dialysate Ca level from 1.75 or 1.50 mmol/L to 1.25 mmol/L was restricted to patients with PTH levels ≤ 300 pg/ml. The study population had quite low PTH levels (mean $=85 \pm 71$ pg/ml), and the majority of them had low-turnover bone disease. Therefore, the favorable results with the reduction of dialysate Ca level from 1.75 or 1.50 mmol/L to 1.25 mmol/L may not be applicable to the entire patient population with HD, and we cannot claim that such a reduction in dialysate Ca level produces better outcomes in patients with PTH levels >300 pg/ml and those without low bone turnover disease. Although dialysate containing 1.25 mmol/L Ca is currently widely used, especially in the United States, an earlier study reported worsening of hyperparathyroid bone disease with reduction of dialysate Ca level from 1.75 to 1.25 mmol/L, despite concomitant use of Ca-based phosphate binders and

vitamin D.³⁹ We, therefore, did not enroll patients with PTH levels >300 pg/ml. Although patients with PTH levels ≤ 300 pg/ml constituted the majority of our dialysis population at the time that this study was designed (use of 1.75–1.50 mmol/L dialysate Ca in $>95\%$ of the patients and PTH levels ≤ 300 pg/ml in 88% of the patients), we cannot claim that the study population represents the general HD population worldwide. In fact, it may not be reasonable to recommend the same dialysate Ca concentration for all patients. Third, we did not measure other serum markers, including fibroblast growth factor 23 level, which has been shown to be associated with VC. Both PTH levels and thus, vitamin D use were higher in the 1.25 Ca group. More vitamin D use in the 1.25 Ca group may increase fibroblast growth factor 23 level and contribute to VC in this group.⁴⁰ However, low doses of vitamin D have been shown to be protective against aortic calcification.⁴¹ As a matter of fact, both high and low levels of calcitriol may be associated with VC.⁴² Fourth, physicians following the enrolled patients in clinics were not blinded; however, investigators who evaluated CAC score and bone histomorphometry, which are the major outcome measures, were blinded.

In conclusion, lowering Ca exposure through dialysate attenuates progression of CAC and improves low bone turnover. A dialysate Ca concentration of 1.25 mmol/L is preferred over 1.75 mmol/L in patients on HD with baseline PTH levels of ≤ 300 pg/ml.

CONCISE METHODS

Study Design and Patients

The Dialysate Calcium Study was a randomized, controlled trial (Clinical Trials ID no. NCT00297219) to evaluate the effects of lowering dialysate Ca concentration from 1.75 or 1.50 mmol/L to 1.25 mmol/L on the progression of CAC and the change in bone turnover, mineralization, and bone volume in patients on HD (Figure 1).

All patients treated in eight dialysis clinics were screened ($n=894$). At the time of screening, $>95\%$ of those patients were treated with a dialysate containing Ca ≥ 1.50 mmol/L; 88% of them had PTH levels ≤ 300 pg/ml, and 11% were treated with active vitamin D. Inclusion criteria were use of a dialysate containing 1.75 or 1.50 mmol/L Ca, age between 18 and 80 years old, conventional HD treatment three times per week for at least 3 months, single pool Kt/V >1.2 , and written informed consent. Exclusion criteria were hypercalcemia (Ca >10.2 mg/dl), PTH values >300 pg/ml, vitamin D treatment within 6 months before randomization, expecting living donor renal transplantation, pregnancy or nursing, mental incompetence, and serious life-limiting comorbid conditions, including active malignancy, chronic infection, and end stage cardiac, pulmonary, or hepatic disease.

In total, 425 subjects met the selection criteria (Figure 1) and were assigned in a 1:1 ratio to either the 1.25- or 1.75-mmol/L dialysate Ca group (1.25 and 1.75 Ca groups, respectively). The study was Institutional Review Board approved and conducted in accordance with the Declaration of Helsinki.

Outcomes

The primary outcome was progression of CAC, and the secondary outcome was changes in histomorphometric parameters of bone turnover, mineralization, and volume. Although the planned follow-up period was 18 months, it was extended to 24 months because of a technical problem in the device used for CAC measurement. CAC measurements and bone biopsies were performed at baseline and the end of the study. Changes in CAC and bone parameters were compared between the 1.25 and 1.75 Ca groups.

All blood samples were taken before a midweek dialysis session and analyzed in a central laboratory. Routine biochemical parameters were measured monthly by an autoanalyzer (Architect c-8000; Abbott Diagnostics, Chicago, IL). Alkaline phosphatase, high-sensitivity C-reactive protein, lipid parameters, and PTH were evaluated quarterly. Intact PTH was determined by the electrochemiluminescence Elecsys PTH Assay (Roche Diagnostics, Indianapolis, IN). The intra- and inter-assay coefficients of variations were 3.8% and 4.2%, respectively.

Except Ca concentration, composition of dialysate was the same and unchanged in the study groups. Reversed osmosis was used in all dialysis clinics. Only Ca-based phosphate binders (Ca acetate or carbonate) were used (a non-Ca-based phosphate binder was not available in the country before the study). Intravenous calcitriol or alfacalcidol was prescribed to control PTH. Treatment strategies were standardized according to KDOQI guidelines.²¹ No calcimimetics were used before or during the study. Subjects were removed from the study if hypercalcemia (>10.2 mg/dl) was found on three sequential measurements.

CAC Scores

CAC scores were measured in all subjects, except those with cardiac arrhythmia or pacemakers, using the same device (Dual Score 64-Slice Multislice Computed Tomography Scanner; Aquilion 16; Toshiba, Tokyo, Japan) and a Ca-scoring program (TeraRecon 3.4.2.11; TeraRecon, Foster City, CA) as described elsewhere.¹⁴ Reported CAC scores were calculated as the sum of scores obtained from the left coronary artery and the right coronary artery. The CAC score was evaluated by two radiologists without knowledge of the study group according to the method by Agatston *et al.*⁴³ Inter- and intraobserver variabilities were 1.2% and 2.5%, respectively. In addition, volume scores were measured, and square roots of the volumes were calculated.⁴⁴

Mineralized Bone Histology and Bone Histomorphometry

Iliac crest bone biopsies were performed after two courses of oral tetracycline hydrochloride.⁴⁵ Bone samples were processed, and histomorphometry was done as published before.^{12,46} Renal osteodystrophy was blindly assessed by evaluating bone turnover, mineralization, and volume.^{46,47}

Statistical Analyses

Sample size was estimated to compare changes in CAC scores in the two groups. We assumed that CAC scores might progress with a rate of 1.27 ± 1.88 per day in the 1.75 Ca group within 18 months as reported previously⁴⁸ and that the use of 1.25 mmol/L dialysate Ca concentration might stop the progression of CAC scores. We estimated that 322 patients would provide 90% power with a two-sided α -error rate

of 5% for detecting a significant difference between the treatment arms. Additionally, progression rates of CAC scores in the two groups were compared. The planned follow-up was 18 months. Considering an estimated dropout rate of 30%, 425 patients were enrolled in the study.

A power analysis for the bone biopsy part of the study was not performed, because there were no data on this subject in the literature. Considering the result of a preliminary questionnaire, it was estimated that only one third of study patients would be willing to undergo bone biopsies.

Data were expressed as means $\pm$ SDs unless otherwise indicated. Baseline and time-averaged parameters (mean of all measurements during follow-up) were compared between the treatment groups by using the *t* test or the Wilcoxon rank sum test on the basis of whether the data were normally distributed and the chi-squared test for categorical variables. Changes in CAC scores and bone parameters from baseline to end of study were compared by paired *t* test. Univariate (Spearman correlation) analyses were done to determine predictors of change in CAC. The absolute difference between the second and first CAC scores (Agatston and volume) and the square root of the volume were assessed.⁴⁴ Sensitivity analyses were also performed using analysis of covariance, with the change in CAC and bone parameters as the dependent variables. Statistical significance was defined as $P < 0.05$. All analyses were done using SPSS (version 13.0; IBM SPSS, Chicago, IL). SAS software (version 9.2; SAS Institute Inc., Cary, NC) was used for covariance analysis to test the interaction between serum phosphorus and dialysate Ca level on progression of CAC score.

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The sponsor had no role in study design and conduction; data management, collection, and analysis; or preparation and submission of the manuscript.

The following investigators participated in the Dialysate Calcium Study: Ali Basci, Meltem Sezis Demirci, Cenk Demirci, Devrim Bozkurt, Hamad Dheir, Savas Sipahi, Ender Hur, (Ege University School of Medicine); Can Boydak, Taskin Colak, (Ege Nefroloji Dialysis Clinic); Burcu Ozmalkoc, (Nefron Dialysis Clinic); Yuksel Yucadag, (Buca Dialysis Clinic); Dilhan Yucadag, (Nefron Dialysis Clinic); Mehmet Sonbahar, (Buca Dialysis Clinic); Banu Kinay, Erdal Karaca, (Sevgi Dialysis Clinic); Ali Ilasslan, Mijgan Sifil, (Nasir Dialysis Clinic); Ertan Bertan, Sermin Odabasioglu, Erdal Sevinc, (Hatay Dialysis Clinic); Sinan Erten, Nurdan Isik, (Narlidere Dialysis Clinic); Yesim Peker, Erhan Lale, and Pinar Uretmen (Gaziemir Dialysis Clinic).

DISCLOSURES

E.O. is a member of the scientific advisory board of Fresenius Medical Care, Turkey. G.A., S.B., H.T., M.O., M.Y., F.K., E.S.O., N.C., S.D., M.C., M.-C.M.-F., and H.H.M. declare that they have no conflicts of interest.

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Supplementary Table 1. Use of calcium-based phosphate binder and vitamin D during the study period in the 1.25 and 1.75 Ca group.

	Months								
	0	3	6	9	12	15	18	21	24
Use of P-binder %									
1.25 Ca group	0.58	0.68	0.67	0.69	0.61	0.64	0.59	0.60	0.58
1.75 Ca group	0.60	0.68	0.63	0.55	0.57	0.61	0.59	0.55	0.52
<i>p value</i>	0.73	0.98	0.35	0.004	0.24	0.37	0.85	0.24	0.30
Use of vit D (%)									
1.25 Ca group	-	0.01	0.03	0.06	0.10	0.12	0.18	0.28	0.38
1.75 Ca group	-	0.01	0.02	0.02	0.01	0.02	0.04	0.08	0.13
<i>p value</i>	-	0.98	0.78	0.10	<0.001	0.002	<0.001	<0.001	<0.001

Supplemental Table 2. Baseline characteristics of patients with and without initial CAC measurement; baseline characteristics of patients with and without second CAC measurement.

	Initial CAC measurement (n=381)	No baseline measurement (n=44)	<i>p value</i>		Two CACS measurement (n=224)	No second measurement (n=157)	<i>p value</i>
Age (years)	59.7 ± 13.7	58.3 ± 13.4	0.52		60.1 ± 13.1	59.2 ± 14.5	0.80
Female (%)	44	41	0.71		43	45	0.56
Diabetes (%)	26.2	20.4	0.40		24.5	28.6	0.37
Time on hemodialysis (months)	50.6 ± 42.2	53.3 ± 35.4	0.68		50.8 ± 41.3	50.2 ± 43.5	0.88
Cardiovascular disease in history (%)	20.5	30.2	0.41		19.8	21.5	0.68
Smoking (%)	29	30	0.85		29	28	0.95
Systolic BP (mmHg)	125 ± 15	128 ± 18	0.23		124 ± 14	126 ± 15	0.31
Diastolic BP (mmHg)	75 ± 9	75 ± 11	0.86		74 ± 9	76 ± 8	0.19
Pre-dialysis urea (mg/dL)	132 ± 33	135 ± 24	0.66		135 ± 35	129 ± 29	0.07
Pre-dialysis creatinine (mg/dL)	8.4 ± 1.9	8.3 ± 2.4	0.59		8.5 ± 2.0	8.2 ± 1.7	0.13
Potassium (mEq/L)	4.9 ± 0.7	4.8 ± 0.6	0.25		4.9 ± 0.7	4.9 ± 0.7	0.91
Calcium (mg/dL)	9.14 ± 0.61	9.22 ± 0.73	0.44		9.15 ± 0.62	9.14 ± 0.62	0.92

Phosphorus (mg/dL)	4.34 ± 1.14	4.31± 1.44	0.89		4.33 ± 0.96	4.35 ± 1.04	0.78
Ca-P product (mg ² /dL ²)	38.0 ± 10.6	38.1 ± 13.5	0.98		38.4 ± 10.3	37.4 ± 11.2	0.42
Alkaline phosphatase (U/L)	103 ± 54	111 ± 72	0.31		98 ± 51	109 ± 57	0.06
Parathyroid hormone (pg/mL)	86 ± 71	80 ± 64	0.63		79 ± 66	95 ± 77	0.03
Equilibrated Kt/V	1.41 ± 0.26	1.35 ± 0.34	0.24		1.39 ± 0.27	1.42 ± 0.24	0.34
Albumin (g/dL)	3.92 ± 0.32	3.91 ± 0.26	0.93		3.91 ± 0.30	3.90 ± 0.35	0.33
Total cholesterol (mg/dL)	181 ± 42	195 ± 51	0.11		182 ± 42	179 ± 42	0.49
Triglyceride (mg/dL)	158 ± 92	211 ± 179	0.06		158 ± 88	158 ± 97	0.95
HDL-cholesterol (mg/dL)	40.2 ± 11.6	42.9 ± 13.2	0.20		40.1 ± 11.0	40.7 ± 12.5	0.49
LDL-cholesterol (mg/dL)	109 ± 35	108 ± 36	0.93		111 ± 35	106 ± 35	0.22
Hemoglobin (g/dL)	11.2 ± 1.2	11.1 ± 1.5	0.72		11.2 ± 1.5	10.9 ± 1.4	0.008
Bicarbonate (mEq/L)	24.2 ± 3.4	25.1 ± 4.0	0.18		24.0 ± 3.5	24.4 ± 3.2	0.25
C-reactive protein (mg/dL)	1.51 ± 1.6	1.23 ± 2.00	0.49		1.50 ± 3.0	1.52± 2.0	0.93
Dialysate Ca before the study			0.68				0.54
1.75 mmol/L (%)	65.2	61.5			66.3	64.2	
1.50 mmol/L (%)	34.8	38.5			33.7	35.8	
Use of P-binder (%)	59	58	0.73		60	57	0.63
Dose of P-binder (elementary Ca,	582 ± 729	698 ± 745	0.27		624 ± 650	572 ± 802	0.34

mg/day)							
CAC score (Agatston)	-	-	-		474 ± 886	705 ± 1508	0.08
CAC score (volume)	-	-	-		384 ± 706	556 ± 1179	0.11

Supplemental Table 3. Baseline characteristics of patients with two CAC measurements in the 1.25 and in the 1.75 mmol/L dialysate Ca groups.

	1.25 Ca group (n=120)	1.75 Ca group (n=104)	<i>p value</i>
Age (years)	60.0 ± 13.3	60.2 ± 12.8	0.91
Female (%)	42	45	0.59
Diabetes (%)	23.3	25.9	0.65
Time on hemodialysis (months)	49.1 ± 37.8	52.9 ± 45.3	0.49
Cardiovascular disease in history (%)	19.3	20.3	0.84
Smoking (%)	31	26	0.38
Systolic BP (mmHg)	124 ± 15	126 ± 14	0.89
Diastolic BP (mmHg)	74 ± 9	75 ± 8	0.68
Pre-dialysis urea (mg/dL)	136 ± 33	134 ± 37	0.74
Pre-dialysis creatinine (mg/dL)	8.7 ± 2.0	8.4 ± 2.0	0.22
Potassium (mEq/L)	4.9 ± 0.7	4.9 ± 0.7	0.90
Calcium (mg/dL)	9.21 ± 0.62	9.10 ± 0.59	0.10
Phosphorus (mg/dL)	4.2 ± 1.0	4.12 ± 1.14	0.73
Ca-P product (mg ² /dL ²)	38.7 ± 10.2	37.5 ± 10.3	0.39

Alkaline phosphatase (U/L)	96 ± 45	100 ± 57	0.54
Parathyroid hormone (pg/mL)	76 ± 68	80 ± 65	0.66
Equilibrated Kt/V	1.38 ± 0.27	1.41 ± 0.28	0.30
Albumin (g/dL)	3.86 ± 0.30	3.87 ± 0.30	0.76
Total cholesterol (mg/dL)	181 ± 43	183 ± 42	0.71
Triglyceride (mg/dL)	157 ± 91	159 ± 85	0.85
HDL-cholesterol (mg/dL)	39 ± 10	40 ± 11	0.52
LDL-cholesterol (mg/dL)	110 ± 35	111 ± 35	0.72
Hemoglobin (g/dL)	11.3 ± 1.4	11.3 ± 1.5	0.98
Bicarbonate (mEq/L)	24.4 ± 3.6	23.6 ± 3.4	0.12
C-reactive protein (mg/dL)	1.46 ± 2.65	1.21 ± 2.07	0.38
Dialysate Ca before the study			
1.75 mmol/L (%)	66.6	65.5	0.76
1.50 mmol/L (%)	33.4	34.5	0.81
Use of P-binder (%)	60	61	0.96
Dose of P-binder (elementary Ca, mg/day)	622 ± 680	627 ± 666	0.95
CAC score (Agatston)	452 ± 869	500 ± 909	0.69
CAC score (volume)	351 ± 679	383 ± 698	0.74

Supplemental Table-4. Baseline characteristics of patients with and without initial bone biopsy; baseline characteristics of patients with and without second bone biopsy.

	Initial bone biopsy (n=185)	No bone biopsy (n=240)	<i>p value</i>		Two bone biopsies (n=108)	No second bone biopsy (n=77)	<i>p value</i>
Age (years)	59.1 ± 13.8	59.9 ± 13.6	0.53		58.1 ± 12.6	60.5 ± 15.2	0.23
Female (%)	48	41	0.15		44	52	0.31
Diabetes (%)	28.6	23.0	0.18		27.7	29.8	0.75
Time on hemodialysis (months)	47.6 ± 37.4	53.4 ± 44.2	0.16		46.1 ± 32.3	49.6 ± 44.2	0.53
Cardiovascular disease in history (%)	22.5	20.4	0.60		23.5	21.0	0.68
Smoking (%)	33.5	25.2	0.07		36.7	28.9	0.27
Systolic BP (mmHg)	124 ± 15	126 ± 16	0.26		124 ± 15	124 ± 15	0.92
Diastolic BP (mmHg)	74 ± 8	75 ± 0	0.21		74 ± 8	74 ± 8	0.95
Pre-dialysis urea (mg/dL)	134 ± 35	132 ± 29	0.43		135 ± 39	132 ± 30	0.56
Pre-dialysis creatinine (mg/dL)	8.4 ± 1.9	8.3 ± 1.9	0.39		8.6 ± 2.0	8.2 ± 1.8	0.11
Potassium (mEq/L)	4.9 ± 0.7	4.8 ± 0.7	0.14		4.9 ± 0.7	5.0 ± 0.8	0.43
Calcium (mg/dL)	9.13 ± 0.64	9.16 ± 0.61	0.64		9.11 ± 0.65	9.16 ± 0.62	0.64
Phosphorus (mg/dL)	4.02 ± 1.06	4.22 ± 1.24	0.10		4.11 ± 1.08	3.90 ± 1.04	0.19

Ca-P product (mg ² /dL ²)	37.0 ± 10.1	38.8 ± 11.5	0.12		37.8 ± 10.0	36.0 ± 10.2	0.23
Alkaline phosphatase (U/L)	103 ± 52	103 ± 59	0.99		102 ± 49	104 ± 56	0.81
Parathyroid hormone (pg/mL)	81.5 ± 70.0	88.0 ± 71.9	0.37		79.9 ± 72.6	83.7 ± 66.6	0.72
Equilibrated Kt/V	1.38 ± 0.24	1.40 ± 0.29	0.39		1.38 ± 0.25	1.39 ± 0.22	0.76
Albumin (g/dL)	3.91 ± 0.32	3.86 ± 0.32	0.10		3.93 ± 0.28	3.89 ± 0.37	0.48
Total cholesterol (mg/dL)	185 ± 44	179 ± 42	0.12		185 ± 43	186 ± 47	0.95
Triglyceride (mg/dL)	164 ± 107	163 ± 104	0.85		167 ± 90	161 ± 127	0.73
HDL-cholesterol (mg/dL)	41.1 ± 11.9	40.1 ± 11.7	0.36		38.7 ± 9.7	44.4 ± 13.8	0.001
LDL-cholesterol (mg/dL)	112 ± 34	106 ± 35	0.11		114 ± 34	109 ± 34	0.32
Hemoglobin (g/dL)	11.2 ± 1.49	11.1 ± 1.48	0.43		11.3 ± 1.5	11.0 ± 1.4	0.25
Bicarbonate (mEq/L)	24.2 ± 3.4	24.3 ± 3.6	0.63		23.8 ± 3.2	24.7 ± 3.5	0.07
C-reactive protein (mg/dL)	1.28 ± 2.07	1.64 ± 2.87	0.16		1.20 ± 1.94	1.40 ± 2.25	0.51
Dialysate Ca before the study			0.74				0.71
1.75 mmol/L (%)	68.1	65.2			67.1	69.1	
1.50 mmol/L (%)	31.9	34.8			32.9	30.9	
Use of P-binder (%)	63.2	59.4	0.64		60.1	64.4	
Dose of P-binder (elementary Ca, mg/day)	693 ± 642	598 ± 623	0.71		680 ± 679	753 ± 815	0.51
CAC score (Agatston)	447 ± 899	645 ± 1357	0.13		433 ± 880	466 ± 932	0.81

CAC score (volume)	362 ± 707	518 ± 1068	0.11		360 ± 696	366 ± 726	0.95
Bone formation rate/Bone surface (normal: 1.80-3.80mm ³ /cm ² /year)	-	-	-		1.22 ± 1.48	1.16 ± 1.40	0.77
Mineralization lag time (normal: <100 days)	-	-	-		138 ± 144	160 ± 272	0.57
Bone volume/Tissue volume (normal: 16.8-22.9%)	-	-	-		18.7 ± 7.1	18.7 ± 7.0	0.99

Supplemental Table 5. Baseline characteristics of patients with two bone biopsies in 1.25 and 1.75 mmol/L dialysate Ca groups.

	1.25 Ca group (n=55)	1.75 Ca group (n=53)	<i>p value</i>
Age (years)	58.1 ± 12.8	58.1 ± 12.5	0.99
Female (%)	36	53	0.08
Diabetes (%)	29.0	26.4	0.75
Time on hemodialysis (months)	47.1 ± 32.7	45.1 ± 32.1	0.74
Cardiovascular disease in history (%)	14.8	32.6	0.03
Smoking (%)	37.0	36.5	0.95
Systolic BP (mmHg)	123 ± 14	125 ± 15	0.69
Diastolic BP (mmHg)	74 ± 7	74 ± 9	0.76
Pre-dialysis urea (mg/dL)	132 ± 37	139 ± 40	0.38
Pre-dialysis creatinine (mg/dL)	8.7 ± 2.0	8.6 ± 2.0	0.77
Potassium (mEq/L)	4.8 ± 0.7	5.0 ± 0.7	0.29
Calcium (mg/dL)	9.17 ± 0.59	9.06 ± 0.71	0.38
Phosphorus (mg/dL)	4.04 ± 0.98	4.18 ± 1.17	0.49
Ca-P product (mg ² /dL ²)	37.3 ± 9.6	38.3 ± 10.5	0.58
Alkaline phosphatase (U/L)	99 ± 44	106 ± 53	0.45

Parathyroid hormone (pg/mL)	83± 72	76 ± 72	0.57
Equilibrated Kt/V	1.40 ± 0.22	1.35 ± 0.28	0.32
Albumin (g/dL)	3.95 ± 0.29	3.90 ± 0.27	0.31
Total cholesterol (mg/dL)	188 ± 44	183 ± 42	0.53
Triglyceride (mg/dL)	167 ± 94	167 ± 86	0.99
HDL-cholesterol (mg/dL)	38 ± 8	39 ± 11	0.73
LDL-cholesterol (mg/dL)	116 ± 37	112 ± 32	0.58
Hemoglobin (g/dL)	11.4 ± 1.3	11.2 ± 1.6	0.56
Bicarbonate (mEq/L)	24.2 ± 3.4	23.4 ± 3.0	0.20
C-reactive protein (mg/dL)	1.16 ± 2.01	1.23 ± 1.89	0.85
Dialysate Ca before the study			
1.75 mmol/L (%)	69.1	70.1	0.72
1.50 mmol/L (%)	30.9	29.9	0.68
Use of P-binder (%)	62.9	67.9	0.59
Dose of P-binder (elementary Ca, mg/day)	633 ± 683	727 ± 678	0.47