

A Bimodal Association of Vitamin D Levels and Vascular Disease in Children on Dialysis

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ABSTRACT

In addition to its classical role in calcium-phosphate homeostasis, vitamin D has anti-inflammatory effects that may influence vascular disease. This study examined the impact of vitamin D levels on the vascular phenotype in 61 children who had been on dialysis for ≥ 3 mo and in 40 age-matched control subjects. All dialysis patients were prescribed daily oral $1\text{-}\alpha$ hydroxyvitamin D₃. 92% of patients were deficient in 25-hydroxyvitamin D [25(OH)D]. 1,25-dihydroxyvitamin D [1,25(OH)₂D] levels were low in 36% and high in 11% of patients. There was a weak correlation between 1α -hydroxyvitamin D₃ dosage and 1,25(OH)₂D levels. Both carotid intima-media thickness and calcification scores showed a U-shaped distribution across 1,25(OH)₂D levels: patients with both low and high 1,25(OH)₂D had significantly greater carotid intima-media thickness ($P < 0.0001$) and calcification ($P = 0.0002$) than those with normal levels. Low 1,25(OH)₂D levels were associated with higher high-sensitivity C-reactive protein ($P < 0.0001$). Calcification was most frequently observed in patients with the lowest 1,25(OH)₂D and the highest high-sensitivity C-reactive protein. In contrast, 25(OH)D levels did not correlate with any vascular measure. In conclusion, both low and high 1,25(OH)₂D levels are associated with adverse morphologic changes in large arteries, and this may be mediated through the effects of 1,25(OH)₂D on calcium-phosphate homeostasis and inflammation. For optimization of strategies to protect the vasculature of dialysis patients, careful monitoring of 1,25(OH)₂D levels may be required.

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Cardiovascular disease is the most common cause of death in patients with chronic kidney disease (CKD).¹ Structural and functional abnormalities and calcification in the large vessels begin as early as the first decade of life,^{2,3} contributing to a 30-fold higher mortality¹ than in the age-matched population. In patients with CKD, vitamin D deficiency and disorders in mineral metabolism associated with secondary hyperparathyroidism are the most important causes of vascular damage and calcification^{4–7} and are also associated with abnormal bone turnover states; this is called CKD-mineral and bone disorder.⁸

Because the failing kidney cannot convert the “nutritional” 25-hydroxyvitamin D₃ [25(OH)D] to the biologically active or “hormonal” form 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D],⁴ 1,25(OH)₂D deficiency is widely prevalent in patients with

CKD.^{9,10} Thus, vitamin D analogues are routinely used, but both vitamin D deficiency and supplementation with vitamin D analogues have been implicated as potential risk factors in the development and progression of vascular disease.^{6,7,11–16}

Vitamin D analogues increase calcium (Ca) and also phosphate (PO₄) absorption and suppress parathyroid hormone (PTH) secretion *via* the vita-

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min D receptor on the parathyroid gland.^{4,17,18} Inadequate 1,25(OH)₂D levels would be expected, therefore, to result in low plasma Ca and hyperparathyroidism, whereas high 1,25(OH)₂D levels cause hypercalcemia, hyperphosphatemia, and oversuppression of PTH. In turn, high bone turnover (as a result of hyperparathyroidism) results in an efflux of Ca and PO₄ from the bones into the soft tissues,⁴ whereas low bone turnover (as a result of oversuppression of PTH) results in an inability of the bone to buffer fluxes in serum Ca and PO₄.^{13,17,18} Thus, it is known that optimal 1,25(OH)₂D levels are required to manipulate PTH so as to achieve effective parathyroid gland suppression and allow a normal bone turnover, but the effects of 1,25(OH)₂D on the vasculature are poorly understood.

In addition to its endocrine effects, vitamin D has important autocrine/paracrine roles: The vitamin D receptor is ubiquitous¹⁹ and, importantly, is present in the vascular smooth muscle cells (VSMC),²⁰ cardiomyocytes,^{9,21} and cells of the monocyte/macrophage lineage.²² 1,25(OH)₂D has important anti-inflammatory and immunomodulatory effects.^{15,23,24} Because inflammation plays a key role in vascular damage,^{25,26} vitamin D may have antiatherogenic effects. Thus, although vitamin D exerts potentially deleterious procalcific effects on the vasculature, its anti-inflammatory properties may confer a significant cardioprotective benefit. In this study, we hypothesize that 1,25(OH)₂D levels in the normal range are associated with less vascular damage and calcification in children on dialysis.

RESULTS

The clinical and biochemical characteristics of the patient and control groups are shown in Tables 1 and 2. Of the 61 patients

(37 boys), 39 had renal dysplasia, nine had inherited nephropathies, five had cystic kidney disease, four had primary renal tubular disorders, three had renovascular disorders, and one had Wilms' tumor.

Vitamin D Levels in Patients and Control Subjects

Levels of 25(OH)D were low in the majority of patients as well as control subjects (Table 3). Despite that all of the patients were prescribed daily oral alphacalcidol, 1,25(OH)₂D levels were low (<40 pmol/L) in 22 (36%) and high (>150 pmol/L) in 11 (18%) patients. 1,25(OH)₂D levels showed a weak correlation with both the alphacalcidol dosage at the time of the study ($P = 0.06$, $r = 0.25$) and the mean time-averaged alphacalcidol dosage ($P = 0.06$, $r = 0.19$). No correlation was found between 25(OH)D and 1,25(OH)₂D levels ($P = 0.4$, $r = 0.11$).

Vitamin D Levels and Their Clinical and Biochemical Correlations

Patients on peritoneal dialysis (PD; $n = 43$, 70%) had significantly lower 1,25(OH)₂D levels than those on hemodialysis (HD; 46.7 ± 18.8 versus 68.2 ± 35.7 pmol/L; $P = 0.02$, $r = 0.31$); 53% of PD and 24% of HD patients had low 1,25(OH)₂D levels. There was no correlation with serum albumin levels in the overall cohort, but PD patients with low 1,25(OH)₂D had lower serum albumin at the time of the study (35.2 ± 8.0 versus 41.1 ± 3.1 ; $P = 0.04$) than those with 1,25(OH)₂D in the normal or high range.

1,25(OH)₂D showed a linear correlation with both the Ca-PO₄ product at the time of the study ($P = 0.02$, $r = 0.28$) as well as the mean time-averaged Ca-PO₄ product ($P = 0.03$, $r = 0.22$; Table 2). Patients with high 1,25(OH)₂D had more hypercalcemic episodes (15 versus 6%; $P = 0.02$). As classically

Table 1. Demographic, clinical, anthropometric, and biochemical characteristics of patients and control subjects^a

Characteristic	Patients (n = 61)	Control Subjects (n = 40)	P
Age (yr; mean ± SD)	13.4 ± 4.1	14.4 ± 3.8	0.2900
Gender (male/female)	37/24	22/18	0.1900
Race (white/Asian/black/other)	37/12/9/3	27/11/2/0	–
eGFR (ml/min per 1.73 m ² ; mean ± SD)	8.9 ± 8.0	121.0 ± 5.8	<0.0001
Time in stage 4 CKD (yr; median [range])	4.9 (0.2 to 6.8)	–	–
Time on dialysis (yr; median [range])	1.10 (0.25 to 8.70)	–	–
Dialysis modality (PD/HD)	43/18	–	–
BMI SDS (mean ± SD)	−0.5 ± 1.6	0.9 ± 0.9	<0.0001
SBP index (mean ± SD) ^b	1.3 ± 0.3	0.9 ± 0.1	0.0200
No. on antihypertensive medications	11	0	–
No. on ACEi or ARB	2	0	–
Hemoglobin (g/dl; mean ± SD)	11.7 ± 1.5	13.3 ± 1.1	0.0700
Albumin (g/L; mean ± SD)	38.0 ± 3.0	41.0 ± 0.6	0.2200
Total cholesterol (mmol/L; mean ± SD)	4.0 ± 1.1	3.3 ± 1.3	0.1000
Triglycerides (mmol/L; mean ± SD)	1.2 ± 1.2	0.9 ± 2.2	0.6700
No. on statins	2	0	–
Diabetes	0	0	–
Smokers	0	1	–

^aARB, angiotensin II receptor blocker; ACEi, angiotensin-converting enzyme inhibitor; BMI, body mass index; SBP, systolic BP; SDS, SD score.

^bBP index = measured BP/95th centile BP for age, gender, and height.

Table 2. Serum Ca, PO₄, and PTH levels and dosages of PO₄ binders and alphacalcidol in patients and control subjects^a

Parameter	Patients (n = 61)		Control Subjects (n = 40)	P ^b
	Exposure from the Onset of Stage 4 CKD	Values at the Time of Study		
Serum PO ₄ level (mmol/L; mean ± SD)	1.5 ± 0.7 ^c	1.4 ± 0.5	0.9 ± 0.3	0.007
Serum Ca (albumin adjusted; mmol/L; mean ± SD)	2.4 ± 0.1 ^c	2.4 ± 0.0	2.3 ± 0.2	0.190
Serum Ca-PO ₄ product (mmol ² /L ² ; mean ± SD)	4.2 ± 0.9 ^c	4.3 ± 0.4	3.3 ± 0.3	0.002
Serum iPTH (fold ULN; mean ± SD)	1.8 ± 1.3 ^c	1.6 ± 0.9	ND	–
PO ₄ binders				
Patients on Ca-based PO ₄ binders (n [%])	NA	52 (88)	–	–
Patients on sevelamer ± Ca-based PO ₄ binders (n [%])		9 (12)	–	
Intake of elemental Ca from PO ₄ binders (g/kg per yr; mean ± SD)	31.9 ± 11.8 ^d	NA	0	–
dosage (mg/day; mean ± SD)	NA	1828 ± 52	0	–
Alphacalcidol (μ/kg per yr; mean ± SD)	19.2 ± 7.3 ^d	NA	0	–
dosage (μ g/d; mean ± SD)	NA	0.89 ± 0.3	0	–
Parathyroidectomy	0	0	0	–

^aND, not done.^bCompares values at the time of the study between patients and control subjects.^cAll biochemical values in this column are expressed as mean time-averaged values from the onset of stage 4 CKD.^dThe dosage of elemental calcium intake from PO₄ binders and the alphacalcidol dosage are expressed as the cumulative intake from stage 4 CKD and standardized per year of exposure to account for the varied duration since onset of stage 4 CKD.

observed, the mean time-averaged intact PTH (iPTH) level inversely correlated with 1,25(OH)₂D [$P = 0.02$, $r = 0.36$ for mean time-averaged iPTH *versus* 1,25(OH)₂D levels and $P = 0.003$, $r = 0.41$ for iPTH levels at the point of study *versus* 1,25(OH)₂D levels]. No correlation was found between 25(OH)D or 1,25(OH)₂D levels and the serum Ca, PO₄, or alkaline phosphatase measured at any time point. Neither 25(OH)D nor 1,25(OH)₂D showed any correlation with the patients' age, gender, race, dialysis vintage, BP, or body mass index. There was no seasonal variation in the levels.

Vitamin D Levels and Correlations with Vascular Measures

Both low and high levels of 1,25(OH)₂D were associated with abnormal vascular measures: Patients with 1,25(OH)₂D in the normal range had carotid intima-media thickness (cIMT) levels comparable to those in the control subjects (Table 3), but those with 1,25(OH)₂D <40 or >150 pmol/L had significantly higher cIMT ($P < 0.0001$ for quadratic term; Figure 1A). The cardiac calcification score showed a similar relationship to 1,25(OH)₂D: Calcification was seen in eight (36%) of 22 pa-

Table 3. Comparison of vascular measures and vitamin D levels between dialysis and control groups

Parameter	Dialysis (n = 61)	Control Subjects (n = 40)	P
cIMT (mm; mean ± SD)	0.48 ± 0.17	0.38 ± 0.01	0.0010
Aortic PWV (m/s; mean ± SD)	7.7 ± 1.0	ND	–
No. with cardiac calcification (%)	13 (21)	ND	
Agatston score (median [range])	141.2 (0.0 to 2039.0)	–	–
coronary arteries	12	–	
valves	3	–	
aorta	2	–	
25(OH)D (ng/ml; mean ± SD) ^a	13.3 ± 10.9	28.2 ± 9.9	<0.0001
<10 (n [%])	30 (50)	0	
10 to 30 (n [%])	26 (42)	29 (72)	
>30 (n [%])	5 (8)	11 (27)	
1,25(OH) ₂ D (pmol/L; median [range])	10 (10 to 216)	ND	
<40 (n [%])	22 (36)		
40 to 150 (n [%])	28 (46)		
>150 (n [%])	11 (18)		

^aTo convert 25(OH)D levels to nmol/L, multiply by 2.5.

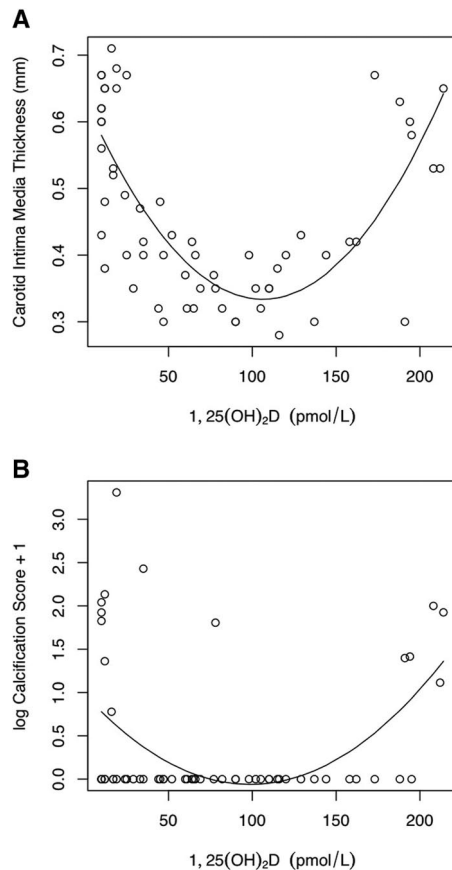


Figure 1. (A) The cIMT showed a significant quadratic relationship with $1,25(\text{OH})_2\text{D}$ levels ($P < 0.0001$). (B) The cardiac calcification score showed a similar significant quadratic relationship ($P = 0.0002$).

tients with $1,25(\text{OH})_2\text{D}$ levels < 40 pmol/L, five (45%) of 11 patients with levels > 150 pmol/L, and only one (3.6%) of 28 patient with levels in the normal range ($P = 0.0002$ for quadratic term; Figure 1B). No association was found between $1,25(\text{OH})_2\text{D}$ and pulse wave velocity (PWV) or between $25(\text{OH})\text{D}$ and any of the vascular measures.

Vitamin D Levels and Correlations with Inflammation

The high-sensitivity C-reactive protein (hs-CRP) level was higher in patients than in control subjects (median 9.3 [range 1.2 to 53] versus 0.43 mg/L [0.1 to 5.02]; $P < 0.00001$, $r = 0.73$) and higher in HD than in PD patients (17.9 [1.7 to 54.4] versus 3.2 mg/L [0.3 to 23]; $P = 0.01$, $r = 0.52$). None of the patients had active infections at the time of the study. The hs-CRP was significantly higher in patients with calcification (15.3 [0.5 to 54.4] versus 1.35 mg/L [0.3 to 32]; $P < 0.0001$, $r = 0.25$; Figure 2) and was independent of age, dialysis vintage, BP, body mass index, lipid profile, cIMT, or PWV. An inverse correlation was seen between $1,25(\text{OH})_2\text{D}$ and hs-CRP ($P < 0.0001$, $r = -0.29$; Figure 3). Both the prevalence of calcification and the highest calcification scores were found in patients with a combination of low $1,25(\text{OH})_2\text{D}$ levels and high hs-CRP, whereas patients with $1,25(\text{OH})_2\text{D}$ levels in the normal range had the

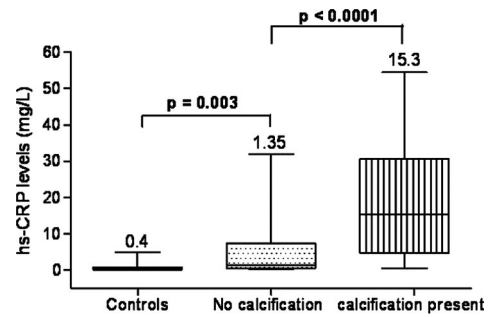


Figure 2. The hs-CRP level was higher in the patients than in the control subjects ($P < 0.00001$, $r = 0.73$) and was highest in the patients with calcification than in those without calcification on CT scan ($P < 0.0001$, $r = 0.25$). Error bars represent the median and range for each group.

lowest incidence of calcification (Figure 4). There was no interaction between $1,25(\text{OH})_2\text{D}$ and hs-CRP ($P = 0.11$). There was no association between $25(\text{OH})\text{D}$ and hs-CRP.

Predictors of cIMT and Calcification

In addition to the strong quadratic relationship between cIMT and $1,25(\text{OH})_2\text{D}$ and between the calcification score and $1,25(\text{OH})_2\text{D}$, we found univariate associations between cIMT and the mean time-averaged Ca-PO_4 product ($r = 0.38$, $P < 0.001$), calcification score and the mean time-averaged Ca-PO_4 product ($r = 0.27$, $P = 0.02$), and calcification score and iPTH levels ($r = 0.18$, $P = 0.04$). Significant predictors of the outcome variables, cIMT and calcification score, from univariate analyses were entered into stepwise multiple linear regression analyses (Table 4). $1,25(\text{OH})_2\text{D}$ was an independent predictor of cIMT, whereas both $1,25(\text{OH})_2\text{D}$ and hs-CRP were significant predictors of the calcification score.

DISCUSSION

This is the first study to show that both low and high levels of $1,25(\text{OH})_2\text{D}$ are associated with abnormal vascular structure

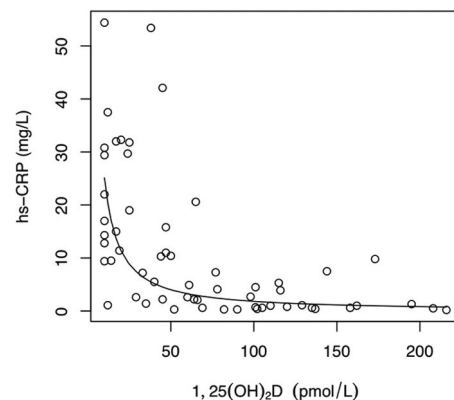


Figure 3. There was an inverse correlation between $1,25(\text{OH})_2\text{D}$ and hs-CRP levels ($P < 0.0001$, $r = -0.29$). The regression line was fitted on the log-log scale and is shown back-transformed.

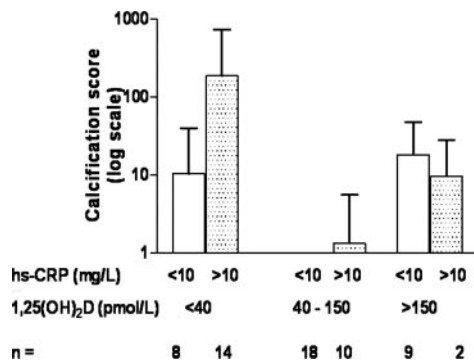


Figure 4. Both the prevalence of calcification and the highest calcification scores were found in patients with a combination of low 1,25(OH)₂D levels and high hs-CRP, whereas patients with 1,25(OH)₂D levels in the normal range had the lowest incidence of calcification.

and calcification, possibly through a dual effect on Ca-PO₄ homeostasis and inflammation. A significant number of children on dialysis have low levels of 1,25(OH)₂D despite daily alphacalcidol supplements, and given the narrow therapeutic window for vitamin D analogues on vascular health, careful monitoring of 1,25(OH)₂D levels is recommended in the dialysis population.

A total of 92% of our patients had 25(OH)D deficiency, and studies have shown that the majority of patients with CKD have low 25(OH)D levels^{9,27}; however, unlike other studies in which the patients were naive to vitamin D sterols, all of our patients were prescribed daily alphacalcidol. The “nutritional” form, 25(OH)D, is unaffected by alphacalcidol treatment, so we also measured 1,25(OH)₂D, the “hormonal,” or “active,” form of vitamin D produced by 25-hydroxylation of alphacalcidol in the liver,^{17,18} and found that 36% of the children had low 1,25(OH)₂D. Reduced levels of 1,25(OH)₂D despite treatment may be due to a loss of albumin-bound vitamin D in the peritoneum, as suspected in our patients, or nonconcordance with treatment. None of our patients had malabsorption syndromes or liver disorders to suggest poor absorption or reduced enzymatic conversion of alphacalcidol. Despite the

strong inverse correlation between inflammation and vitamin D and the higher inflammatory status of HD patients, we found lower vitamin D levels in the PD cohort, suggesting that a loss of albumin-bound vitamin D did indeed play a major role. Earlier work from our group showed that in children on dialysis, the dosage of alphacalcidol influenced cIMT and calcification,³ but the lack of a consistent dosage-response relationship as shown in this study suggests that adjustments in the alphacalcidol dosage alone may not be sufficient for optimal management.

The effects of high vitamin D dosages on vascular calcification are widely recognized in clinical studies^{3,16,28–30} and *in vitro* work,^{20,31–33} and this study confirms an increased cIMT and calcification in patients with high 1,25(OH)₂D levels. Vitamin D analogues may induce calcification by a number of mechanisms that include enhancing the gastrointestinal absorption of Ca and PO₄, oversuppression of PTH leading to adynamic bone disease,^{13,17,18} and a direct effect on VSMC.^{20,31–33} An early study by Milliner *et al.*³⁴ showed that, at autopsy, 60% of children with CKD had soft tissue calcification and 36% had systemic calcinosis, and use of any vitamin D analogue showed the strongest independent association with calcinosis. In addition, both 25(OH)D and 1,25(OH)₂D can have a direct effect on the VSMC: 1,25(OH)₂D upregulates the vitamin D receptor and induces cellular calcium uptake,³¹ decreases VSMC proliferation,²⁰ and induces VSMC migration and osteoblastic conversion of the VSMC.^{32,33} Recent observational studies by Litwin *et al.*,²⁹ Civilibal *et al.*,²⁸ and Mitsnefes *et al.*³⁰ and our own work³ have shown that a high vitamin D dosage adversely affects cIMT^{3,29,30} and calcification.^{3,28} Newer vitamin D analogues, such as paricalcetriol and doxercalciferol, are shown to be less calcemic^{35,36} but have only a marginal survival advantage over calcitriol,^{11,12} and their effects on the vasculature have not as yet been studied.

Despite the clearly deleterious calciotropic effects of vitamin D, in this study, low levels of 1,25(OH)₂D were also associated with increased cIMT and calcification. Low 1,25(OH)₂D results in an increase in PTH levels that can promote soft tissue calcification through its effect on calcium absorption and an efflux of Ca and PO₄ from a high-turnover bone state.^{13,17,18}

Table 4. Multivariate analysis for predictors of cIMT and calcification score

Variables	β^a	SE	P	Model R ^{2b}
cIMT				57%
1,25(OH) ₂ D level	−2.49	0.001	<0.0001	
square of 1,25(OH) ₂ D level (quadratic coefficient)		<0.001	<0.0001	
mean time-integrated Ca-PO ₄ product	0.32	0.090	0.0730	
Calcification score ^c				16%
1,25(OH) ₂ D level	−9.10	4.200	0.0400	
square of 1,25(OH) ₂ D level (quadratic coefficient)	0.04	0.020	0.0400	
hs-CRP	11.60	4.700	0.0200	
mean time-integrated Ca-PO ₄ product	10.20	1.700	0.1600	
iPTH levels	5.20	2.600	0.4800	

^aUnstandardized regression coefficient; indicates the difference in the outcome variable (cIMT or calcification score) per unit change in the independent variables.

^bThe amount of variance in the dependent variable that can be explained by the model.

^cAnalyzed as log_e(calcification score + 1).

Our findings are supported by two studies of adult HD patients that demonstrated a approximately 20% survival advantage of any vitamin D formulation over no vitamin D treatment.^{11,12} In a small cohort of Japanese HD patients, Shoji *et al.*³⁷ showed that the use of 1- α hydroxyvitamin D₃ was associated with a 70% lower risk for death from cardiovascular disease as compared with a group not on any vitamin D supplements. London *et al.*³⁸ recently showed that in a cohort of adult dialysis patients who all were naive to vitamin D analogues, low 25(OH)D and 1,25(OH)₂D were associated with greater vessel stiffness and reduced brachial artery distensibility. In an earlier study, we showed that children who were on dialysis and had PTH levels more than two-fold the upper limit of normal (ULN) had higher cIMT, PWV, and calcification than those with PTH levels less than two-fold the ULN³; however, as seen in this study, 1,25(OH)₂D levels were associated with cIMT and calcification independent of PTH, suggesting that the biologic consequences of 1,25(OH)₂D extend beyond the regulation of Ca-PO₄ homeostasis alone.

Anti-inflammatory actions of vitamin D^{22,26} may also contribute to its effects on the vasculature but have not been explored in clinical studies. Interestingly, we found a strong inverse correlation between hs-CRP and 1,25(OH)₂D levels that was associated with vascular calcification but no association between 25(OH)D and hs-CRP. 1- α Hydroxylase is expressed on many cell types such as macrophages, endothelial cells, and dendritic cells, where it acts in an autocrine/paracrine manner independent of the PTH–bone axis and is unaffected by renal failure but may be regulated by immune stimuli. *In vitro* studies as well as studies in other inflammatory disease states, such as rheumatoid arthritis,³⁹ have shown that vitamin D can influence various aspects of inflammation,⁴⁰ including inhibition of antigen-presenting cell maturation, downregulation of NF- κ B, and modulation of cytokine production to create an anti-inflammatory environment^{22,41} (increased IL-10 and decreased IL-6, IL-12, and TNF- α), but this is the first clinical study that has found an association between 1,25(OH)₂D levels and inflammation; however, it is also possible that inflammation leads to low vitamin D levels. In a cross-sectional study, it would be impossible to discern a cause–effect relationship between inflammation–malnutrition and vitamin D levels, but given the results of *in vitro* studies that have shown a causal effect of vitamin D on inflammation, it is likely that low vitamin D levels contribute to the proinflammatory milieu in dialysis patients. Studies of patients without renal failure have shown that vitamin D supplementation can suppress serum TNF- α and increase IL-10 levels⁴²; TNF- α promotes atherosclerosis and IL-10 has antiatherogenic properties. Vitamin D also has direct cardioprotective effects such as an antiproliferative effect on cardiomyocytes²¹ and negative endocrine regulation of the renin-angiotensin system.⁴³

In this cross-sectional study, an association between vitamin D levels and vascular phenotype does not necessarily indicate a cause–effect relationship; however, this study serves as a starting point to stimulate cell biology work and generate

hypotheses for randomized, controlled studies of the effects of vitamin D analogues on cardiovascular health. We were unable to find a correlation between hs-CRP levels and cIMT, suggesting that either the study population was too small to demonstrate an effect or inflammation has a greater influence on calcification than on the mechanisms involved in vessel thickening. Unlike the study by London *et al.*,³⁸ we did not find a correlation between vitamin D levels and vessel stiffness. The greater plasticity of children's vessels and their shorter dialysis vintage may allow for compensatory mechanisms that can maintain normal vessel function in the face of early structural damage to the vessel. Vessels from children provide an ideal model to study uremic influences on the arterial wall, because they do not have the confounding proatherosclerotic risk factors that are prevalent in the adult CKD population.

In conclusion, we have shown, for the first time, that both low and high levels of 1,25(OH)₂D are associated with adverse morphologic changes in the large arteries and that the vascular damage may be determined by the effects of vitamin D on Ca-PO₄ homeostasis and inflammation. Given the narrow therapeutic window for vitamin D analogues on vascular health, optimal vascular protective strategies in dialysis patients may require careful monitoring of not only the vitamin D dosage but also 1,25(OH)₂D levels.

CONCISE METHODS

Study Design

From January 2005 to December 2006, 61 consecutive children (5 to 18 yr) who had been on dialysis for ≥ 3 mo were recruited. Levels of 25(OH)D, 1,25(OH)₂D and hs-CRP were measured and related to cIMT, PWV, and the presence of coronary and valvular calcification on multislice computed tomography (CT) scan. Patients were compared with healthy age- and gender-matched school children who underwent vascular scans in our department as part of a parallel study investigating normal levels of cIMT and PWV in healthy children. From this cohort, we included 40 consecutive children aged 5 to 18 yr with no known medical illnesses or family history of heart disease or diabetes to serve as control subjects in our study, in a 1.5:1 patient:control subject ratio. Of note, the majority of children in the control group were overweight, although no intentional selection bias was involved. Informed written consent was obtained from all parents or caregivers and from children when appropriate. The study was approved by the local research ethics committee.

In our center PD is offered to all children as the initial dialysis modality unless specific contraindications such as recent intra-abdominal surgery, peritoneal infections, or difficult family circumstances preclude it. As a result, our group of patients on HD are often older and have a longer dialysis vintage. All patients in this study were prescribed daily oral 1- α hydroxycholecalciferol (alphacalcidol), titrating the dosage so as to keep the iPTH level less than two-fold the ULN. Hyperphosphatemia was managed by dietary PO₄ restriction and calcium-based PO₄ binders. Sevelamer, a newer non-calcium-

based PO₄ binder, was used only for patients with hypercalcemia and persistently high iPTH levels.

Data Collection

Serum Ca, PO₄, and iPTH levels and the dosages of elemental calcium intake from phosphate binders and alphacalcidol were recorded at monthly intervals from the start of stage 4 CKD, and mean time-averaged values were calculated (the sum total of the monthly values for each variable was divided by the number of months of exposure to provide a mean time-averaged value). Given the variable amounts of time spent in stage 4 CKD, the biochemical values and medication dosages at the time of the study are expressed separately in Table 2. Because the iPTH assay has changed over time, iPTH is expressed as multiples of the ULN. The number of hypercalcemic episodes (defined as albumin-adjusted serum Ca levels >2.5 mmol/L and expressed as a percentage of the total number of albumin-adjusted serum Ca measurements in each patient) was calculated. In control subjects, a single blood test at the time of the scans was performed.

Vitamin D Levels and hs-CRP

All biochemical data were determined on blood samples taken before the vascular scans and before a midweek session of HD but at varying times throughout the year. There was an interval of 10 to 12 h between alphacalcidol intake and blood sampling. Serum 25(OH)₂D and 1,25(OH)₂D levels were measured by a single technician using the enzyme immunoassay (IDS-OCTEIA 25-HydroxyvitaminD EIA kit; Immunodiagnosics Systems Ltd., Tyne and Wear, UK) and RIA (1,25 dihydroxyvitamin D ¹²⁵I RIA kit; DiaSorin, Stillwater, MN), respectively. 1,25(OH)₂D levels in 120 healthy normal children have been measured and validated in our laboratory (unpublished data), so we measured 25(OH)₂D levels only in the 40 control subjects. hs-CRP levels were determined by ELISA (EIA test kit; MP Biomedicals, Orangeburg, NY) for all patients and control subjects.

Carotid Ultrasonography for cIMT

B-mode ultrasound of both common carotid arteries was performed using a 12-MHz linear array transducer (Vivid 7; GE Medical, Horton, Norway). Longitudinal two-dimensional images of the vessel 1 to 2 cm proximal to the carotid bulb were acquired on the R wave of the electrocardiogram (ECG), frozen in diastole, and analyzed off-line. The cIMT was calculated as the distance between the leading edge of the lumen-intima interface and the media-adventitia interface on the far wall of the artery.

Applanation Tonometry for PWV

Applanation tonometry in the carotid and femoral arteries was performed with a micromanometer (SPC-301; Millar Instruments, Houston, TX) and recorded directly onto a computer running proprietary software (SphygmoCor version 7.0; Scanmed, Gloucestershire, UK). An ECG gated signal and standard distance measurements were used. PWV was computed from contour analysis of 10 consecutive pressure waveforms recorded consecutively in the carotid and femoral arteries to give the aortic PWV. PWV was not measured in the control subjects because permission was not granted for femoral artery measurements.

Multislice-Spiral CT for Cardiac Imaging

Sixteen-slice spiral CT scans were performed (Somatom Sensation 16; Siemens Medical Solutions, Erlangen, Germany) using the standard Ca scoring protocol. Prospective ECG triggering was performed to obtain image acquisition in diastole, using 60% of the RR interval for image reconstruction. The Agatston score⁴⁴ for each main epicardial coronary artery, cardiac valves, and aorta and an overall score for each patient were determined. In the control group, CT was not undertaken because of radiation concerns.

Statistical Analyses

Results are presented as means ± SD or median and range, depending on the distribution. Comparisons between groups were made using *t* test or the Mann-Whitney *U* test as appropriate, and correlations were tested using Pearson or Spearman correlation tests for parametric and nonparametric data, respectively. The two main outcome variables were cIMT and calcification score [transformed to log₁₀(CS + 1) to adjust for skewness]. Factors affecting the two outcome variables were explored using multiple regression analysis. From univariate analyses, variables with *P* < 0.15 were entered into the stepwise multiple regression analyses. The nonlinear effect of vitamin D levels was fitted by including a quadratic term: The outcome variables were regressed on 1,25(OH)₂D and the square of 1,25(OH)₂D, and the significance of the squared (quadratic) term was noted. A third outcome measure, hs-CRP, was analyzed after log transformation to account for skewness, and its correlation with 1,25(OH)₂D was strengthened when that, too, was log-transformed. The interaction between 1,25(OH)₂D and hs-CRP was tested by two way ANOVA. *P* ≤ 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 12.0.1 (SPSS, Chicago, IL).

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DISCLOSURES

None.

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