ORIGINAL RESEARCH

Impact of Cholecalciferol Treatment on Biomarkers of Inflammation and Myocardial Structure in Hemodialysis Patients without Hyperparathyroidism

Sérgio Bucharles, MD, PhD,[★†] *Silvio Henrique Barberato, MD, PhD,*[★‡] *Andréa E. M. Stinghen, PhD,*[★§] *Betina Gruber,*[★] *Luciana Piekala, MD,*[†] *Ana Cláudia Dambiski, MD,*[†] *Melani R. Custodio, MD, PhD,*[¶] *and Roberto Pecoits-Filho, MD, PhD*[★]

Introduction: Vitamin D (25-hydroxyvitamin D, 25(OH)D) deficiency, hypovitaminosis D, is highly prevalent in chronic kidney disease patients and is potentially involved with complications in the hemodialysis (HD) population. The aim of this study was to evaluate the impact of cholecalciferol supplementation on biomarkers of mineral metabolism, inflammation, and cardiac function in a group of HD patients presenting with hypovitaminosis D and low intact parathyroid hormone (iPTH) levels.

Material and Methods: HD patients with iPTH levels of <300 pg/mL, not receiving vitamin D therapy, and presenting with 25(OH)D levels of <30 ng/mL were enrolled in this prospective study. Oral cholecalciferol was prescribed once a week in the first 12 weeks (50,000 IU) and in the last 12 weeks (20,000 IU) of the study. High-sensitivity C-reactive protein, interleukin-6, and serum albumin were used as inflammatory markers. Echocardiograms were performed on a midweek interdialytic day at baseline and after 6 months of cholecalciferol supplementation.

Results: In all, 30 patients were included in the final analysis. We observed a significant increase in serum 25(OH)D levels after 3 months (46.2 ± 14.4 ng/mL vs. 18.1 ± 6.6 ng/mL; $P < .001$) and after 6 months (40.4 ± 10.4 ng/mL vs. 18.1 ± 6.6 ng/mL; $P < .001$) of cholecalciferol supplementation. There were no significant changes in alkaline phosphatase, iPTH, phosphorus, and serum albumin levels, but there was a slight but significant increase in calcium levels after 6 months of cholecalciferol supplementation (9.4 ± 0.6 mg/dL vs. 9.0 ± 0.6 mg/dL; $P = .02$). Additionally, we observed a significant reduction in high-sensitivity C-reactive protein levels after 3 months (median: 0.62 [0.05 to 29.6] mg/L vs. 0.32 [0.02 to 3.13] mg/L; $P = .02$) and after 6 months (median: 0.62 [0.05 to 29.6] mg/L vs. 0.50 [0.02 to 5.66] mg/L; $P = .04$) of cholecalciferol supplementation, as well as a significant reduction in interleukin-6 levels (median: 6.44 pg/mL vs. 3.83 pg/mL; $P = .018$) after 6 months of supplementation. Left ventricular mass index was significantly reduced at the end of supplementation (159 ± 55 g/m^2 vs. 175 ± 63 g/m^2; $P = .03$).

Conclusions: Cholecalciferol supplementation in HD patients was found to be safe and efficient to correct hypovitaminosis D and established little impact on mineral metabolism markers. Additionally, we observed a reduction in important surrogate markers of cardiovascular risk, namely systemic inflammation and left ventricular hypertrophy, suggesting an anti-inflammatory action and possibly an improvement of cardiac dysfunction.

★*Renal Division, Center for Health and Biological Sciences, Pontificia Universidade Católica do Paraná, Curitiba, Paraná, Brazil.*

†*Dialysis Division, Instituto do Rim do Paraná, Curitiba, Paraná, Brazil.*

‡*Echocardiographic Division, Hospital Cardiológico Costantini, Curitiba, Paraná, Brazil.*

§*Basic Pathology Department, Universidade Federal do Paraná, Curitiba, Brazil.*

¶*Renal Division, Universidade Federal de Uberlandia, Brazil.*

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Address reprint requests to Roberto Pecoits-Filho, MD, PhD, Renal Division, Center for Health and Biological Sciences, Pontificia Universidade Católica do Paraná, Rua Imaculada Conceição 1155, Curitiba, Paraná 80215-901, Brazil. E-mail: r.pecoits@pucpr.br

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CARDIOVASCULAR DISEASE (CVD) is a major cause of morbidity and mortality in chronic kidney disease (CKD) population,[1] and although traditional cardiovascular risk factors (hypertension, diabetes mellitus, dyslipidemia) are frequent in these patients, the pathogenesis of CVD involves the interplay of traditional risk factors and uremia-related factors, such as mineral metabolism disorders[2] and systemic inflammation.[3]

Hypovitaminosis D (vitamin D deficiency) is frequently observed among CKD patients, particularly in those on hemodialysis (HD) treatment.[4-6] Vitamin D receptors (VDRs) are found ubiquitously throughout the body, and most tissues and many cells possess the enzymatic mechanisms (1-alpha hydroxylase) to convert vitamin D into its active form, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$; calcitriol). Indeed, an activated VDR exhibits classic actions related to bone and mineral metabolism and other so-called noncalcemic actions, especially in the cardiovascular system and many different cells of immune system,[7,8] potentially acting as a cell differentiating factor and antiproliferative agent.[9] In fact, hypovitaminosis D has been recently associated with higher risk of CVD in CKD and in the general population.[10-12]

Bone and mineral metabolism guidelines for CKD patients recommend the measurement of 25-hydroxyvitamin D (25(OH)D) levels in patients with CKD stages 3 to 5 and those on dialysis, independently of intact parathyroid hormone (iPTH) levels, and replacement therapy with ergocalciferol or cholecalciferol in those who present with hypovitaminosis D.[13] However, there is no consistent background information supporting the need for screening hypovitaminosis D in the dialysis population with low iPTH and its association with asymptomatic CVD and inflammation. Moreover, the impact of hypovitaminosis D correction with cholecalciferol on left ventricular hypertrophy (LVH) and inflammation, both surrogate markers of CVD, has not been consistently demonstrated in the literature, particularly in patients with low levels of iPTH.

Thus, the aims of this study were to evaluate the effects of oral cholecalciferol supplementation on mineral metabolism parameters, inflammation biomarkers, and echocardiographic variables in CKD HD patients with hypovitaminosis D and low iPTH levels.

Materials and Methods

Study Design

This was a prospective study of stable HD patients from a single renal replacement therapy center performed during fall and winter of 2010 in the city of Curitiba, Brazil.

Population

A total population of 384 HD patients was screened for the study. History of CVD, iPTH levels of >300 pg/mL, and utilization of vitamin D supplementation or analogs were used as exclusion criteria. History of CVD (coronary artery disease, chronic heart failure, and valvular diseases) was an exclusion criterion to avoid the interference of previous cardiac disease on the analysis of the myocardial impact of cholecalciferol. Similarly, patients with high iPTH levels were excluded to isolate the known effects of parathyroid hormone on the myocardium. Additionally, we excluded patients with inflammatory conditions, including malignances, chronic infections, and autoimmune diseases. Of the original population, 45 patients fulfilled the inclusion and exclusion criteria.

All patients underwent dialysis sessions with low-flux polysulfone membranes, and the dialysate calcium concentration was 3.5 meq/L during the study. Dialysis dose was delivered to achieve a Kt/V of >1.2. Hemoglobin levels were monitored to achieve K/DOQI-recommended parameters, and all patients were on epoetin-alpha therapy. The target level for ferritin was between 200 ng/mL and 800 ng/mL, and intravenous iron saccharate was used when needed. Patients who were lost to follow-up were also excluded from final analysis (8 patients underwent renal transplantation, 2 patients died from cardiovascular causes, 4 patients changed to peritoneal dialysis, and 1 patient was transferred to another dialysis facility), and after a 6-month period, we were able to analyze the baseline and final data from 30 patients.

Systolic blood pressure and diastolic blood pressure were measured during midweek HD sessions before beginning the study and in the last month of cholecalciferol supplementation. Pulse pressure was calculated using the following formula: PP = SBP − DBP (where PP = pulse pressure, SBP = systolic blood pressure, DBP = diastolic blood

pressure), on the basis of blood pressure evaluation shortly before the HD sessions. Antihypertensive therapy, especially use of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers, as well as therapy with statins, was evaluated before and after cholecalciferol supplementation.

Oral cholecalciferol (Farmadoctor Pharmacy, Curitiba, Brazil) was prescribed once a week in the first 12 weeks (50,000 IU) and in the last 12 weeks (20,000 IU) of the study. To monitor compliance, dialysis nurses administered cholecalciferol immediately after HD sessions.

Biochemical Analysis

All the biochemical variables were measured before beginning supplementation therapy and at the end of the study (6-month period observation). Blood samples to measure serum calcium, phosphorus, alkaline phosphatase, hemoglobin, and albumin levels and to determine Kt/V were obtained immediately before the first midweek dialysis session in the beginning and during the last midweek dialysis session in the last month of the study.

Serum 25(OH)D level was determined by chemiluminescence method (DiaSorin LIAISON 25OH Vitamin D assay, Diasorin Inc. Stillwater, Minnesota),[14] with intra-assay and interassay coefficients of variability of, on average, 4% and 6%, respectively. The normal range used for 25(OH)D was 30 ng/mL to 60 ng/mL, and levels >150 ng/mL were considered to be in the toxic range. Total iPTH (1-84) was evaluated by a radioimmunoassay, with normal values ranging from 12 pg/mL to 65 pg/mL. Serum albumin level was measured using a colorimetric assay. Interleukin-6 (IL-6) level was measured by the Enzyme Linked Immuno Sorbent Assay technique, and high-sensitivity C-reactive protein (hs-CRP) level was assessed by nephelometry.[3]

Echocardiographic Analysis

Echocardiograms were performed on the midweek interdialytic day, between 8 A.M. and 1 P.M., as previously recommended.[15] The same experienced cardiologist (S.H.B.) performed all examinations using a commercially available ultrasound system (Philips Envisor CD Ultrasound, Phillips Inc., Andover, Massachusetts) equipped with a 2.5-MHz transducer. The echocardiographer was blinded to patients' clinical and laboratory conditions (before and after cholecalciferol administration). According to the Penn convention,[16] linear measurements were obtained from M-mode calculations. The left ventricular mass index (LVMI) was calculated using the Devereux formula[17] and indexed to body surface area. The combination of LVMI and relative wall thickness (2 × mean wall thickness/LV diastolic diameter, where LV = left ventricular) defined 4 LV geometric *patterns:* normal *geometry, concentric* remodeling, eccentric LVH, and concentric LVH. Relative wall thickness reference cutoff value of 0.45[18,19] separated eccentric (below) from concentric (above) LVH. Concentric remodeling was defined as normal LV mass plus an increased relative wall thickness. Ejection fraction was calculated by Simpson's method, and Doppler mitral flow velocities were recorded from the apical 4-chamber view, as recommended by the American Society of Echocardiography.[18] Ejection fraction of <55% was considered as being diagnostic of systolic dysfunction.

Peak early (E) and atrial (A) transmitral velocities, E/A ratio, and deceleration time of early diastolic filling were measured. Time doppler imaging (TDI) of mitral annular velocities were obtained with a small (2 mm) sample volume placed sequentially at the septal and lateral junctions of the LV wall with the mitral annulus.[20] Early (E') and late (A') diastolic mitral annular velocities, E'/A' ratio, and E/E' ratio presented in our study represent the mean value between the two sites. All velocities and intervals were averaged over 3 cardiac cycles. Diastolic dysfunction was defined by: (1) E/A of <1; (2) E/A of >2; or (3) E/A of between 1 and 2, with concomitant E/E' of >10. Left atrial volume was determined through 2-dimensional biplane Simpson's method.[19] Measurements were made at end systole and indexed to both body surface area (BSA) (left atrial volume index (LAVi)–BSA) and height$^{2.7}$ (LAVi–height$^{2.7}$).[21] Normal LAVi–BSA has been determined to be 22 ± 6 mL/m^2; however, a cutoff value of 32 mL/m^2 was indicative of major cardiovascular risk.[22]

Statistical Analysis

All tests were performed using JMP Windows 8.0 (SAS Institute Inc., Cary, North Carolina). Categorical variables were expressed as frequencies, mean values with standard deviation for

Table 1. Clinical and Biochemical Parameters Before and After 6 Months of Cholecalciferol Supplementation (N = 30)

Clinical and Biochemical Characteristics	Baseline	6 Months	*P*
Age (mean ± SD/years)	59 ± 15		
Female gender (%)	53		
Diabetic (%)	33		
Time on HD (median: range, months)	23 (4–60)		
ACEIs (%)	56	60	N/S
ARBs (%)	3	3	N/S
Antihypertensive therapy (%)	96	96	N/S
SBP (mm Hg; mean ± SD)	139 ± 16	138 ± 20	N/S
DBP (mm Hg; mean ± SD)	82 ± 8	83 ± 9	N/S
Pulse pressure (mm Hg; mean ± SD)	57 ± 12	54 ± 14	N/S
Dry weight (kg; median: range)	69 (48–88)	69 (48–90)	N/S
Hemoglobin (g/dL; mean ± SD)	11.7 ± 1.4	12.4 ± 1.4	N/S
Kt/V	1.4 ± 0.3	1.4 ± 0.3	N/S
Epoetin-alpha (IU/kg/week; median: range)	122 (31–247)	120 (44–250)	N/S
Statin therapy (%)	56	56	N/S

SD, standard deviation; HD, hemodialysis; ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; SBP, systolic blood pressure; DBP, diastolic blood pressure; SD, standard deviation; Kt/V, eKt/V; N/S, not significant.

normally distributed variables, and median values with interquartile ranges for non-normally distributed variables. Comparison among variables at baseline, 3 months, and 6 months of cholecalciferol supplementation was performed with analysis of variance or Friedman repeated measure analysis. Comparison between variables at baseline and after 6 months of supplementation was performed using Student paired *t* test or Wilcoxon signed rank test. We considered $P < .05$ to be statistically significant.

Results

The study population included 30 patients with mean age of 59 ± 15 years, 53% females, and on HD for a median of 23 (range: 4 to 60) months. Ten patients (33%) had diabetes, and all but one patient were on antihypertensive therapy. The baseline clinical and biochemical characteristics of our study population are reported in Table 1.

After 6 months of cholecalciferol supplementation, there were no changes in hemoglobin levels, use of antihypertensive therapy, statins and epoetin-alpha use, as well as systolic, diastolic, and pulse pressures (Table 1).

Moreover, we observed a significant increase in serum 25(OH)D levels after 3 months (18.1 ± 6.6 ng/mL vs. 46.2 ± 14.4 ng/mL; $P < .001$) and after 6 months (18.1 ± 6.6 ng/mL vs. 40.4 ± 10.4 ng/mL; $P < .001$; Table 2 and Fig. 1) of cholecalciferol supplementation, with most patients presenting with normal 25(OH)D. There was no significant change in alkaline phosphatase, iPTH, and phosphorus levels, but there was a significant increase in calcium levels after 6 months of cholecalciferol supplementation, compared with baseline values (9.0 ± 0.6 mg/dL vs. 9.4 ± 0.6 mg/dL; $P = .02$;

Table 2. Mineral Metabolism Parameters Before and After 6 Months of Cholecalciferol Supplementation (N = 30)

Biochemical Parameters	Baseline	6 Months	*P*
25(OH)D (ng/mL; mean ± SD)	18.1± 6.6	40.4 ± 10.4	<.001
Calcium (mg/dL; mean ± SD)	9.0 ± 0.6	9.4 ± 0.6	.02
Phosphorus (mg/dL; mean ± SD)	4.8 ± 1.1	5.1 ± 1.7	N/S
Alkaline phosphatase (IU/L; mean ± SD)	78.1 ± 29.6	80.5 ± 36.6	N/S
Intact parathyroid hormone (pg/mL; mean ± SD)	165 ± 80	184 ± 139	N/S

25(OH)D, 25-hydroxyvitamin D.

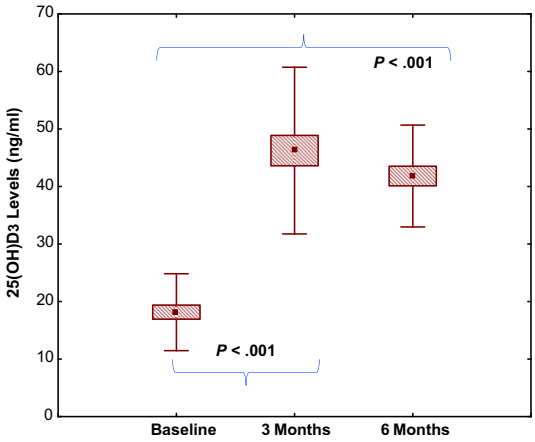

Figure 1. 25-hydroxyvitamin D levels at baseline and after 3 and 6 months of cholecalciferol supplementation (N = 30).

Table 2). Only 2 patients presented with calcium levels of ≥10.5 mg/dL after 3 months of supplementation, and 1 patient presented with calcium levels of 10.5 mg/dL after 6 months of supplementation.

Additionally, we observed a significant reduction in hs-CRP levels after 3 months (median: 0.62 [0.05 to 29.6] mg/L vs. 0.32 [0.02 to 3.13] mg/L; $P = .02$) and after 6 months (median: 0.62 [0.05 to 29.6] mg/L vs. 0.50 [0.02 to 5.66] mg/L; $P = .04$; Table 3) of cholecalciferol supplementation, as well as a significant reduction in IL-6 levels (median: 6.44 [1.36 to 19.58] pg/mL vs. 3.83 [0.78 to 19.45] pg/mL; $P = .018$) after 6 months of cholecalciferol supplementation (Table 3 and Fig. 2). There was no change in serum albumin levels before and after supplementation (Table 3).

At echocardiographic evaluation, we observed that LVMI was significantly reduced at the end of supplementation (175.1 ± 63.1 g/m^2 vs. 159.0 ± 55.2 g/m^2; $P = .03$; Fig. 3 and Table 4). There were no significant changes in mean systolic and diastolic LV diameters, as well as in relative wall thickness. We also observed no significant changes in the prevalence of systolic and diastolic dysfunction, as well in valvular calcification, when we compared values obtained at baseline with those obtained after 6 months of cholecalciferol supplementation.

Discussion

CVD is the main cause of death in CKD patients,[23] and many traditional and nontraditional risk factors, including disturbances of mineral metabolism, are involved in the pathogenesis of CVD in uremia.[24] In the present study, we observed that HD patients with hypovitaminosis D and low iPTH levels presented with a reduction in biomarkers of inflammation and in LVMI after 6 months of supplementation with cholecalciferol.

Hypovitaminosis D is a very common finding in HD population,[6] in patients not on dialysis therapy,[25] as well as in the general population.[26] The interest in studying the mineral effects of supplementation with cholecalciferol in CKD patients with low vitamin D levels has increased after 2 recent observations: first, although kidneys are the primary site for hydroxylation of vitamin D by 1-alpha hydroxylase, this enzyme was localized in a wide variety of tissues that present with the enzymatic machinery to produce 1,25(OH)$_2$D, pointing to the importance of vitamin D status, especially in the cardiovascular and immune systems. Second, several studies demonstrated an association between hypovitaminosis D and cardiovascular risk in CKD patients and in the general population[11,12] by mechanisms that are not fully understood. Indeed, these mechanisms may involve inadequate VDR activation in the cardiovascular tissue[8,26] and the immune system. Because immune dysfunction and CVD are extremely common in the CKD population,[24] the potential benefits of vitamin D supplementation may be greater than in the other group of patients.

The replacement of 25(OH)D in CKD HD patients has been previously studied,[27-29] focusing on safety, effectiveness, and effects on mineral

Table 3. Inflammation Biomarkers Before and After 6 Months of Cholecalciferol Supplementation (N = 30)

Inflammation Biomarkers	Baseline	6 Months	*P*
Serum albumin (mean ± SD)	4.2 ± 0.4	4.3 ± 0.3	N/S
hs-CRP (mg/L; median: range)	0.62 (0.05-29.6)	0.5 (0.02-5.66)	.04
IL-6 (pg/mL; median: range)	6.44 (1.36-19.58)	3.83 (0.78-19.45)	.018

hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6.

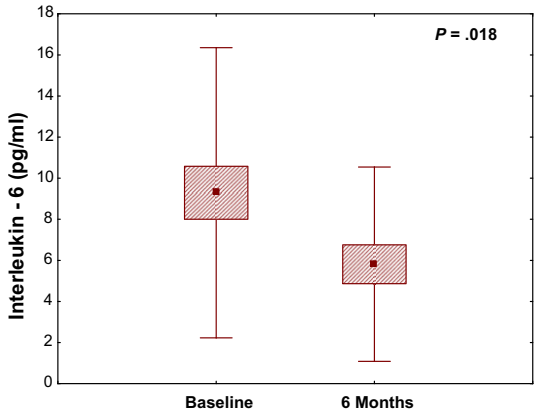

Figure 2. Interleukin-6 levels at baseline and after 6 months of cholecalciferol supplementation (N = 30).

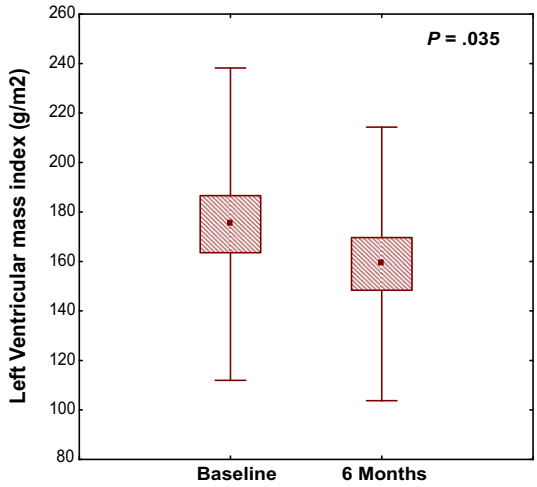

Figure 3. Left ventricular mass index (g/m^2) at baseline and after 6 months of cholecalciferol supplementation (N = 30).

metabolism parameters. Similar to previous reports,[29] we observed that cholecalciferol was efficient in normalizing serum 25(OH)D levels and showed no major side effects with regard to mineral metabolism, although we observed that 2 patients and 1 patient showed hypercalcemia (corrected total calcium level of >10.4 mg/dL) after 3 months and after 6 months of cholecalciferol supplementation, respectively.

There are descriptions of associations between systemic inflammation and vitamin D deficiency in experimental studies. Vitamin D (calcitriol) could potentially induce a better profile of cytokine network, decreasing the expression of IL-6, interleukin-1, and interferon-gamma and promoting upregulation of the anti-inflammatory cytokine interleukin-10.[30-32] In humans, systemic inflammation is frequently observed in dialysis patients, and it is a significant predictor of mortality in this population.[33] Although multiple causes are most likely involved, hypovitaminosis D represents an unrecognized (and potentially reversible) factor playing a role in the generation of this inflammatory state. Our results showed a significant reduction in hs-CRP and IL-6 levels after cholecalciferol supplementation, which is in agreement with previous reports.[34,35] This could reflect an anti-inflammatory effect of vitamin D and could potentially represent a new therapeutic opportunity to reduce systemic inflammation and mortality in CKD HD patients.

LVH is the most frequent cardiovascular abnormality in HD patients and is a strong predictor of mortality in this population.[36,37] The pathogenesis of LVH has been extensively studied in uremia, and although traditional risk factors such as fluid overload, hypertension, and anemia are involved, they cannot fully explain the changes observed in the uremic myocardium.

Table 4. Echocardiographic Parameters at Baseline and After 6 Months of Cholecalciferol Supplementation (N = 30)

Echocardiographic Parameters	Baseline	6 Months	*P*
LVMI (g/m^2; mean ± SD)	175.1 ± 63.1	159.0 ± 55.2	.03
Systolic dysfunction* (%)	10	13	N/S
Diastolic dysfunction† (%)	80	76	N/S
Valvular calcification (%)	26	30	N/S
Relative wall thickness	0.48 ± 0.1	0.48 ± 0.08	N/S
Systolic left ventricular diameter (mm)	32 ± 5	35 ± 4	N/S
Diastolic left ventricular diameter (mm)	51 ± 5	50 ± 4	N/S

LVMI, left ventricular mass index.
*Systolic dysfunction = ejection fraction (by Simpson's method) of <55%.
†Diastolic dysfunction = E/A of <1; (2) E/A of >2; or (3) E/A of between 1 and 2, with concomitant E/E' of >10.

Hypovitaminosis D potentially plays a significant role in the myocardiopathy related to CKD, as $1,25(OH)_2D$ acts as a negative regulator of renin–angiotensin synthesis, which induces inflammatory changes in the myocardium, leading to hypertrophy and fibrosis.[38,39] In our study, we observed a significant reduction in LVH after 6 months on cholecalciferol treatment. Indeed, there are previous studies that observed the impact of $1,25(OH)_2D$[40,41] and cholecalciferol on myocardial mass and function,[34,42] but these studies included patients with several degrees of myocardial dysfunction, including patients with coronary artery disease and those with secondary hyperparathyroidism, who were receiving concomitant $1,25(OH)_2D$ therapy.

In CKD patients, excessive iPTH influences cardiovascular structure and function,[43] inducing LVH through cardiomyocyte hypertrophy and interstitial fibrosis. In some clinical studies, cholecalciferol therapy induced a reduction in LVH in parallel to a decrease in iPTH levels.[34,42] The present study sheds light in this area, isolating the deleterious action of iPTH in the myocardium, as the studied patients presented at the baseline with already low iPTH levels (<300 pg/mL), which were not affected by vitamin D supplementation. Indeed, this is the first report of a reduction in LV mass in a selected group of patients with low levels of iPTH (supposedly without hyperparathyroidism) and not receiving any other form of vitamin D, suggesting a direct effect of 25(OH)D on myocardial cells, where it can act as an antiproliferative and cell differentiating factor.[8,44]

Our study has some limitations, as it did not include a control group, had a relatively low number of patients, and presented a short time of follow-up to capture echocardiographic changes. However, maintaining patients with hypovitaminosis D in placebo treatment would present ethical issues. Also, selecting a population with low iPTH levels and without concurrent vitamin D therapy and no previous CVD definitely limits the recruitment of eligible patients. However, this highly selected population reinforces the principle that vitamin D repletion may have direct benefits on myocardial structure, independent from calcium, phosphorus, and parathyroid hormone levels. We believe that this highly selected population indeed represents an opportunity to analyze the effects of vitamin D supplementation that are not related to mineral metabolism actions. Further studies will need to address the long-term effects of vitamin D nutritional supplementation on the myocardial function and structure.

In conclusion, cholecalciferol supplementation in HD patients was safe and efficient to correct hypovitaminosis D and demonstrated little impact on mineral metabolism. Additionally, there was a reduction in important surrogate markers of cardiovascular risk, namely systemic inflammation and LVH. This study suggests that vitamin D plays a pivotal role in uremic CVD and its supplementation should be considered even in the absence of mineral metabolism disorders, after hypovitaminosis is detected.

References

1. Foley RN, Parfrey PS. Cardiovascular disease and mortality in ESRD. *J Nephrol*. 1998;11:239-245.
2. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM. Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. *J Am Soc Nephrol*. 2004;15:2208-2218.
3. Honda H, Qureshi AR, Heimburger O, et al. Serum albumin, C-reactive protein, interleukin 6, and fetuin a as predictors of malnutrition, cardiovascular disease, and mortality in patients with ESRD. *Am J Kidney Dis*. 2006;47:139-148.
4. Del Valle E, Negri AL, Aguirre C, Fradinger E, Zanchetta JR. Prevalence of 25(OH) vitamin D insufficiency and deficiency in chronic kidney disease stage 5 patients on hemodialysis. *Hemodial Int*. 2007;11:315-321.
5. Gonzalez EA, Sachdeva A, Oliver DA, Martin KJ. Vitamin D insufficiency and deficiency in chronic kidney disease. A single center observational study. *Am J Nephrol*. 2004;24:503-510.
6. Mucsi I, Almasi C, Deak G, et al. Serum 25(OH)-vitamin D levels and bone metabolism in patients on maintenance hemodialysis. *Clin Nephrol*. 2005;64:288-294.
7. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol*. 2005;289:F8-F28.
8. Andress DL. Vitamin D in chronic kidney disease: a systemic role for selective vitamin D receptor activation. *Kidney Int*. 2006;69:33-43.
9. O'Connell TD, Berry JE, Jarvis AK, Somerman MJ, Simpson RU. 1,25-Dihydroxyvitamin D3 regulation of cardiac myocyte proliferation and hypertrophy. *Am J Physiol*. 1997; 272(4 Pt. 2):H1751-H1758.
10. Al-Aly Z. Vitamin D as a novel nontraditional risk factor for mortality in hemodialysis patients: the need for randomized trials. *Kidney Int*. 2007;72:909-911.
11. London GM, Guerin AP, Verbeke FH, et al. Mineral metabolism and arterial functions in end-stage renal disease: potential role of 25-hydroxyvitamin D deficiency. *J Am Soc Nephrol*. 2007; 18:613-620.
12. Wang TJ, Pencina MJ, Booth SL, et al. Vitamin D deficiency and risk of cardiovascular disease. *Circulation*. 2008; 117:503-511.
13. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int Suppl*. 2009;113:S1-S130.

14. Zerwekh JE. Blood biomarkers of vitamin D status. *Am J Clin Nutr.* 2008;87:1087S-1091S.

15. Parfrey PS, Foley RN, Harnett JD, Kent GM, Murray D, Barre PE. Outcome and risk factors of ischemic heart disease in chronic uremia. *Kidney Int.* 1996;49:1428-1434.

16. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. *Circulation.* 1977;55:613-618.

17. Devereux RB, Alonso DR, Lutas EM, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol.* 1986;57:450-458.

18. Quinones MA, Otto CM, Stoddard M, Waggoner A, Zoghbi WA. Recommendations for quantification of Doppler echocardiography: a report from the Doppler Quantification Task Force of the Nomenclature and Standards Committee of the American Society of Echocardiography. *J Am Soc Echocardiogr.* 2002;15:167-184.

19. Lang RM, Bierig M, Devereux RB, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr.* 2005;18:1440-1463.

20. Sohn DW, Song JM, Zo JH, et al. Mitral annulus velocity in the evaluation of left ventricular diastolic function in atrial fibrillation. *J Am Soc Echocardiogr.* 1999;12:927-931.

21. Pritchett AM, Jacobsen SJ, Mahoney DW, Rodeheffer RJ, Bailey KR, Redfield MM. Left atrial volume as an index of left atrial size: a population-based study. *J Am Coll Cardiol.* 2003; 41:1036-1043.

22. Barberato SH, Pecoits-Filho R. Prognostic value of left atrial volume index in hemodialysis patients. *Arq Bras Cardiol.* 2007;88:643-650.

23. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis.* 1998;32(5 suppl. 3):S112-S119.

24. Stinghen AE, Bucharles S, Riella MC, Pecoits-Filho R. Immune mechanisms involved in cardiovascular complications of chronic kidney disease. *Blood Purif.* 2010;29:114-120.

25. LaClair RE, Hellman RN, Karp SL, et al. Prevalence of calcidiol deficiency in CKD: a cross-sectional study across latitudes in the United States. *Am J Kidney Dis.* 2005;45:1026-1033.

26. Lee JH, O'Keefe JH, Bell D, Hensrud DD, Holick MF. Vitamin D deficiency an important, common, and easily treatable cardiovascular risk factor? *J Am Coll Cardiol.* 2008;52:1949-1956.

27. Tokmak F, Quack I, Schieren G, et al. High-dose cholecalciferol to correct vitamin D deficiency in haemodialysis patients. *Nephrol Dial Transplant.* 2008;23:4016-4020.

28. Saab G, Young DO, Gincherman Y, Giles K, Norwood K, Coyne DW. Prevalence of vitamin D deficiency and the safety and effectiveness of monthly ergocalciferol in hemodialysis patients. *Nephron Clin Pract.* 2007;105:c132-c138.

29. Jean G, Terrat JC, Vanel T, et al. Daily oral 25-hydroxycholecalciferol supplementation for vitamin D deficiency in haemodialysis patients: effects on mineral metabolism and bone markers. *Nephrol Dial Transplant.* 2008;23:3670-3676.

30. Panichi V, De Pietro S, Andreini B, et al. Calcitriol modulates in vivo and in vitro cytokine production: a role for intracellular calcium. *Kidney Int.* 1998;54:1463-1469.

31. Cohen-Lahav M, Douvdevani A, Chaimovitz C, Shany S. The anti-inflammatory activity of 1,25-dihydroxyvitamin D3 in macrophages. *J Steroid Biochem Mol Biol.* 2007;103:558-562.

32. Takahashi K, Horiuchi H, Ohta T, Komoriya K, Ohmori H, Kamimura T. 1 alpha,25-dihydroxyvitamin D3 suppresses interleukin-1beta-induced interleukin-8 production in human whole blood: an involvement of erythrocytes in the inhibition. *Immunopharmacol Immunotoxicol.* 2002;24:1-15.

33. Pecoits-Filho R, Barany P, Lindholm B, Heimburger O, Stenvinkel P. Interleukin-6 is an independent predictor of mortality in patients starting dialysis treatment. *Nephrol Dial Transplant.* 2002;17:1684-1688.

34. Matias PJ, Jorge C, Ferreira C, et al. Cholecalciferol supplementation in hemodialysis patients: effects on mineral metabolism, inflammation, and cardiac dimension parameters. *Clin J Am Soc Nephrol.* 2010;5:905-911.

35. Stubbs JR, Idiculla A, Slusser J, Menard R, Quarles LD. Cholecalciferol supplementation alters calcitriol-responsive monocyte proteins and decreases inflammatory cytokines in ESRD. *J Am Soc Nephrol.* 2010;21:353-361.

36. London GM. Left ventricular alterations and end-stage renal disease. *Nephrol Dial Transplant.* 2002;17(suppl 1):29-36.

37. Silberberg JS, Barre PE, Prichard SS, Sniderman AD. Impact of left ventricular hypertrophy on survival in end-stage renal disease. *Kidney Int.* 1989;36:286-290.

38. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest.* 2002;110:229-238.

39. Achinger SG, Ayus JC. The role of vitamin D in left ventricular hypertrophy and cardiac function. *Kidney Int Suppl.* 2005;95:S37-S42.

40. Lemmila S, Saha H, Virtanen V, Ala-Houhala I, Pasternack A. Effect of intravenous calcitriol on cardiac systolic and diastolic function in patients on hemodialysis. *Am J Nephrol.* 1998;18:404-410.

41. Park CW, Oh YS, Shin YS, et al. Intravenous calcitriol regresses myocardial hypertrophy in hemodialysis patients with secondary hyperparathyroidism. *Am J Kidney Dis.* 1999;33:73-81.

42. Coratelli P, Petrarulo F, Buongiorno E, Giannattasio M, Antonelli G, Amerio A. Improvement in left ventricular function during treatment of hemodialysis patients with 25-OHD3. *Contrib Nephrol.* 1984;41:433-437.

43. Rostand SG, Drueke TB. Parathyroid hormone, vitamin D, and cardiovascular disease in chronic renal failure. *Kidney Int.* 1999;56:383-392.

44. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr.* 2004;79:362-371.