

A Randomized Trial of Vitamin D Supplementation on Vascular Function in CKD.

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ABSTRACT

Background

Vitamin D deficiency is associated with mortality in patients with chronic kidney disease (CKD). Its supplementation might mitigate cardiovascular disease (CVD) risk in CKD. We investigated the effect of cholecalciferol supplementation on vascular function in patients with non-diabetic CKD stage 3-4 and vitamin D deficiency.

Methods

In this randomized, double blind, placebo controlled trial, 120 patients of either sex aged 18-70 years with non-diabetic CKD stage 3-4 and vitamin D deficiency [serum 25(OH)D ≤ 20 ng/ml] were randomized (1:1) to receive either two directly observed oral doses of 300,000 IU of cholecalciferol or matching placebo at baseline and 8 weeks. The primary outcome was change in endothelium dependent brachial artery flow mediated dilatation (FMD) at 16 weeks. Secondary outcome measures included changes in pulse wave velocity (PWV), augmentation index (AI) and circulating biomarkers.

Results

Cholecalciferol supplementation significantly increased endothelium dependent brachial artery FMD whereas no significant change was observed in the placebo group [between-group difference in mean change: 5.49% (95% CI: 4.34 to 6.64, $p < 0.001$)] at 16 weeks. Intervention also led to significant favourable changes in PWV and circulating interleukin-6 (IL-6) levels.

Conclusions

In non-diabetic patients with stage 3-4 CKD and vitamin D deficiency, vitamin D supplementation leads to improvement in vascular function. (Clinical Trials Registry of India number: CTRI/2013/05/003648).

KEYWORDS

Chronic kidney disease, cardiovascular disease, vitamin D, endothelial function, flow mediated dilatation, inflammation

BACKGROUND

Since most patients with early chronic kidney disease (CKD) die of cardiovascular disease (CVD) before developing advanced kidney failure, CVD risk reduction is an important management goal in CKD. The widespread recognition of vitamin D deficiency, and the strong epidemiological data linking vitamin D deficiency to cardiovascular mortality in general population and in subjects with CKD has kindled interest in examination of vitamin D deficiency as a potentially modifiable CVD risk factor.¹ In a meta-analysis, each 10 ng/ml reduction in vitamin D increased the risk of all-cause mortality by 14% in subjects with CKD.² Another meta-analysis showed a 37% reduction of cardiovascular mortality with active vitamin D therapy in subjects with CKD.³

Endothelial dysfunction sets in early in subjects with CKD and correlates with future CVD.^{4, 5} Vitamin D induces concentration dependent increase in endothelial nitric oxide (NO) production.⁶ Low serum 25(OH)D has been shown to be an independent predictor of impaired endothelial function in CKD.⁴ Small observational and case control studies have shown improvement in endothelial function with vitamin D supplementation.^{7, 8} Despite the strong epidemiologic links, clinical trial evidence of impact of native vitamin D supplementation on CVD risk or outcomes in patients with CKD is lacking.

Vitamin D repletion strategies in CKD have largely involved use of activated vitamin D because of the belief that vitamin D activation will be hampered in subjects with CKD, and the greater affinity of VDR for activated forms.⁹⁻¹¹ The recognition that many tissues, including endothelial cells, possess 1- α hydroxylase suggests that correction of native vitamin D

deficiency can lead to therapeutic benefits as a result of endocrine and/or paracrine functions of vitamin D, and has generated interest in therapy with native forms.¹²

Flow mediated dilatation (FMD), a well-established technique for assessing endothelial function,¹³⁻¹⁵ predicts CVD events in patients with CKD.^{5, 16} CVD risk factor modification improves FMD, making it a relevant and accepted surrogate for CVD events.¹³

We investigated whether supplementation with cholecalciferol would improve brachial artery FMD in non-diabetic CKD patients with vitamin D deficiency. We also evaluated changes in pulse wave velocity (PWV), a marker of vascular stiffness; and biomarkers of endothelial function [E-selectin and von Willebrand factor (vWF)] and inflammation [high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6)].

METHODS

Study design

The study was a single centre, parallel arm, randomized, double blind, placebo-controlled trial done at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India. The study was approved by Institute Ethics Committee and registered with Clinical Trials Registry of India in May 2013. Subject enrolment started in July 2013 and was completed in April 2015.

Study population

Stable CKD patients attending the Renal Clinic at PGIMER, Chandigarh and having estimated glomerular filtration rate (eGFR) between 15-60 ml/min/1.73m² by 4 variable MDRD

equation were eligible for screening. Inclusion criteria were age 18-70 years, vitamin D deficiency [serum 25(OH)D levels ≤ 20 ng/ml] and absence of diabetes mellitus. Exclusion criteria were heart failure, present or past malignancy, pregnancy in case of females, or vitamin D (ergocalciferol or cholecalciferol) supplementation within the past 30 days. All subjects provided written informed consent prior to enrolment.

Randomization and blinding

Subjects were randomised in a 1:1 allocation ratio, and entered a 2 week run-in period during which usual treatment was verified and continued. Simple randomization was done by computer generated Bernoulli random number table. Neither the investigator nor the patient were aware of the randomization sequence. Blinding was achieved by opaque, sequentially numbered sealed envelopes. Both the drug and placebo were similar in physical appearance, form and packaging.

Intervention and follow up

Upon completion of the run-in period, subjects received their assigned interventions - two oral doses of either 300,000 IU of cholecalciferol or matching placebo at baseline and 8 weeks later. Both doses were given under direct observation. Follow up visits were done 8 and 16 weeks after the baseline visit. Serum creatinine, calcium, inorganic phosphorus, lipid profile, uric acid, and haemoglobin were measured at baseline and the 16-week visits on fasting blood samples. Serum 25(OH)D was measured by enzyme immunoassay [EIA; Immunodiagnostic Systems (IDS), UK], certified for calculation of 25(OH)D by Center for Disease Control (CDC) Vitamin D Standardization Certification Program (VDSCP).^{17, 18}

Vascular function studies

FMD and endothelium independent nitroglycerine mediated dilatation (NMD) were measured at baseline and 16 weeks.¹⁵ Subjects came in fasting state in the morning and withheld their morning medication dose until after the studies. FMD assessment was done in a quiet room with low ambient lighting and temperature 20-25°C by the same trained investigator using Philips IU22 xMatrix ultrasound system (Philips, USA) and Philips IU22 L12-5 linear transducer with extended operating frequency range of 5-12 MHz.

After a 30-minute rest, a 2-minute baseline recording of the right brachial artery was obtained following which a cuff was tied 2 cm distal to the elbow and inflated to 300 mmHg for 5 minutes. A 5-minute recording of brachial artery was made, starting just before cuff deflation, to estimate FMD. After resting again for 15 minutes, a 2-minute recording was repeated, followed by sublingual administration of 0.4 mg of glyceryl trinitrate (GTN). Starting just before GTN administration, 5-minute recording was obtained again to estimate NMD. Recordings were analysed in a random order by a blinded independent investigator using Brachial Analyzer for Research 6 (Vascular Research Tools 6, Medical Imaging Applications LLC, USA), an automated continuous edge detection and wall tracking software. FMD and NMD were defined as the maximum percentage increase in brachial artery diameter during reactive hyperaemia after cuff release, and after GTN administration, respectively.

Carotid-femoral PWV and augmentation index (AI) were calculated by a trained investigator using SphygmoCor® CPV system (AtCor Medical, Inc., IL, USA) at baseline and 16 weeks on the same day as brachial artery FMD assessment.

Biomarker evaluation

Plasma and serum samples at baseline and 16 week visits were stored at -80°C. Serum 1,25(OH)₂D (EIA, IDS, UK), serum intact parathyroid hormone (iPTH; EIA, Calbiotech Inc., USA), serum intact fibroblast growth factor-23 [FGF-23; second generation enzyme linked immunosorbent assay (ELISA), Immutopics, Diagnostic Hybrids Inc., USA], serum E-selectin (Quantikine® solid phase sandwich ELISA, R&D System, USA), serum hs-CRP (Quantikine® solid phase sandwich ELISA, R&D System, USA), serum IL-6 (Quantikine® solid phase sandwich ELISA, R&D System, USA), and plasma vWF (IMUBIND® ELISA, Sekisui Diagnostic, USA) were analysed in all samples.

Outcomes

The primary outcome was change in brachial artery FMD at 16 weeks. Secondary outcome measures were changes in PWV, AI, and levels of serum 1,25(OH)₂D, serum iPTH, serum FGF-23, serum E-selectin, serum hs-CRP, serum IL-6 and plasma vWF.

Sample size calculation and statistical analysis

Assuming a dropout rate of 10%, a sample size of 120 subjects (60 in each group) was required to detect an absolute mean difference of 1.6% (standard deviation 3%) with 80% power and two sided α of 0.05. Assuming a mean FMD of 4% at baseline and no change in the placebo arm, this corresponds to a 40% relative improvement in the cholecalciferol arm.

All enrolled subjects with non-missing outcome data were included in the analysis. Continuous variables were compared with independent samples Student's t test if normally distributed, or with Mann-Whitney U test if the distribution was skewed. Categorical variables

were analysed by Chi Square test or Fisher's Exact test as appropriate. Paired Student's t-test and Wilcoxon signed-rank test were used for within-group comparisons. ANCOVA was used to determine difference between groups for change in FMD over 16 weeks with adjustment for baseline FMD. Two tailed p-values <0.05 were considered statistically significant. Analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software for Macintosh, version 21.0 (IBM Corp., Armonk, NY, USA) and SAS software (SAS Institute, Cary, NC, USA).

Pre-specified subgroup analyses were conducted using a linear model of FMD change including the subgroup variable (gender, tertiles based on serum 25(OH)D levels at baseline, age <50 years or ≥50 years and use of statins or renin angiotensin system inhibitors), the intervention and the interaction between the intervention and the subgroup variable. Heterogeneity was assessed by testing the interaction term between the intervention and the subgroup variable. Post hoc analyses were correlation of change in FMD with change in serum 25(OH)D levels (Spearman's rank correlation) and comparison of change in FMD/NMD between two groups at 16 weeks.

RESULTS

A total of 423 subjects were screened. After excluding 303 subjects, 120 were enrolled, randomized and received the assigned intervention (figure 1). In the cholecalciferol group, 2 subjects did not come for follow up visits and were excluded from final analyses. In the placebo group, follow up FMD could not be done in one subject and therefore, the subject was excluded from analyses.

Baseline characteristics of the two groups are shown in table 1. There were no differences between groups with respect to demographic details, causes of CKD and concomitant medications. Baseline biochemical parameters, circulating biomarker levels and vascular assessment parameters (table 2) also did not differ between groups.

Change in serum 25(OH)D levels

Serum 25(OH)D levels were similar in both groups at baseline (placebo versus cholecalciferol group: 13.21 ± 4.78 versus 13.40 ± 4.42 ng/ml; $p=0.88$; table 2). At 16 weeks, the serum 25(OH)D levels increased in the cholecalciferol group [between-group difference in mean change: 23.40 ng/ml; 95% CI: 19.76 to 27.06; $p<0.001$; table 3] but not in the placebo group.

Changes in Vascular function

Baseline FMD was similar in both the groups (placebo versus cholecalciferol: 7.85 ± 2.35 versus 7.65 ± 2.25 %; $p=0.65$; table 2). At 16 weeks, the FMD improved in the cholecalciferol but not in placebo group [between-group difference in mean change: 5.49% (95% CI: 4.34 to 6.64, $p<0.001$); table 3]. This change in FMD remained significant after adjustment for baseline FMD ($p<0.001$). In the cholecalciferol group, the mean proportional increase in FMD was 80% (supplementary table S1). Change in FMD remained significant in all prespecified subgroups ($p<0.001$, figure 2) with potential heterogeneity by sex and age. Change in serum 25(OH)D levels significantly correlated with change in FMD ($r=0.567$, $p<0.001$).

NMD and FMD/NMD were also improved in the cholecalciferol group [between-group difference in mean change: 2.85% (95% CI: 1.41 to 4.84, $p<0.001$) and 0.25 (95% CI: 0.05 to 0.46, $p=0.017$) for NMD and FMD/NMD, respectively; table 3]. PWV decreased significantly at

16 weeks in the cholecalciferol group [between-group difference in mean change: -1.24 m/sec, 95% CI: -2.16 to -0.74, $p<0.001$, table 3]. At 16 weeks, PWV in the cholecalciferol group was significantly lower as compared to the placebo group ($p<0.001$; supplementary figure S1).

The blood pressure control remained similar between two groups at 16 weeks, and there was no change in systolic or diastolic blood pressures in either group over the study period.

Change in biochemical parameters and circulating biomarkers

Serum calcium levels increased whereas alkaline phosphatase levels decreased in the cholecalciferol group [between-group difference in mean change: 0.69 mg/dl (95% CI: 0.31 to 1.06, $p=0.001$) and -20.25 U/L (95% CI: -35.14 to -5.38, $p=0.008$) for serum calcium and alkaline phosphatase, respectively; table 3]. Serum inorganic phosphorus (Pi), serum creatinine, eGFR and hemoglobin levels did not change in either group.

Serum 1,25(OH)₂D levels increased whereas iPTH and IL-6 levels decreased in the cholecalciferol group [between-group difference in mean change: 14.98 pg/ml (95% CI: 4.48 to 27.18, $p=0.007$), -100.73 pg/ml (95% CI: -150.50 to -50.95, $p<0.001$), and -2.28 pg/ml (95% CI: -3.96 to -0.38, $p=0.001$) for serum 1,25(OH)₂D, iPTH and IL-6, respectively; table 3].

Adverse events

Supplementary table S2 shows the adverse events recorded during the study. There was no hospitalization or serious adverse event. One subject complained of mild headache and dizziness after NMD testing which improved with 15 minutes of rest.

DISCUSSION

This randomized placebo controlled trial provides the strongest evidence so far that cholecalciferol supplementation improves FMD and PWV in pre-dialysis CKD subjects, both of which are predictors of future CVD risk. Change in serum 25(OH)D level significantly correlated with change in FMD, lending further support to vitamin D deficiency as a potentially modifiable CVD risk factor in subjects with CKD.

This finding builds on existing observational and case control studies that have shown a link between vitamin D levels and endothelial function.^{4, 8} These clinical findings are supported by the observation of a dose dependent rise in endothelial nitric oxide synthase (eNOS) gene expression in cultured human aortic endothelial cells after ergocalciferol supplementation,¹⁹ and are concordant with the PENNY trial, in which a 12-week paricalcitol supplementation caused improvement in FMD in patients with CKD stage 3-4.²⁰

Despite concerns about perceived resistance to the action of native vitamin D in CKD, our observations show that cholecalciferol supplementation has the ability to improve FMD. Since vitamin D deficiency is widespread in CKD, this finding has the potential to change guidelines to recommend supplementation for CVD risk reduction, rather than only for management of mineral and bone disorder.

There are additional novel findings that advance the understanding of mechanism of action of different forms of vitamin D. Cholecalciferol supplementation improved not only FMD, but also NMD. As opposed to paricalcitol, cholecalciferol seems to improve the vascular smooth

muscle responsiveness to NO, reflected in NMD.^{20, 21} Though NMD has traditionally been used as a control test for FMD, association of impaired NMD with established CVD and risk factors of CVD have been reported recently.^{22, 23} In a prospective, observational study, impairment of NMD alone was an independent predictor of future CVD events.²⁴ Recent studies have shown improvement in NMD after cholecalciferol supplementation but have been limited by study design.^{7, 25} The ratio of FMD to NMD has been suggested as an index of endothelial function in the setting of significant change in NMD.²¹ In our study, FMD to NMD ratio changed favourably, indicating modulation of endothelial function by cholecalciferol independent of vascular smooth muscle effects.²¹

PWV, a marker of arterial stiffness and atherosclerotic transformation and an independent predictor of CVD and mortality, is regulated by vessel geometry and material properties of vessel wall, and affected by endothelial as well as smooth muscle functions. Studies of vitamin D supplementation have shown inconsistent results in terms of impact on PWV.^{7, 19, 25}, probably because of problems with study design and inadequate numbers. The improvement in PWV is likely a combination of improved endothelial function and direct vascular smooth muscle effects of vitamin D.

Understanding of the pleiotropic effects of vitamin D has been advanced by the discovery of VDR on a number of tissues including endothelial and vascular smooth muscle cells.^{10, 26} Though circulating 1,25(OH)₂D levels are mainly influenced by renal 1- α -hydroxylase, conversion of 25(OH)D to 1,25(OH)₂D by local 1- α -hydroxylase occurs in many tissues. It has been suggested that endothelial dysfunction in vitamin D deficient state could be related to

decreased expression of local 1- α -hydroxylase and VDR which is dependent on circulating 25(OH)D but independent of circulating 1,25(OH)₂D levels.²⁷

The change in serum iPTH levels achieved in this study with cholecalciferol supplementation is comparable to that reported in PENNY, which goes against the traditional argument favouring the use of activated vitamin D compounds for better suppression of elevated iPTH, at least in early stages of CKD.^{11, 28} Finally, cholecalciferol supplementation was safe, and did not have any adverse effect on eGFR, whereas paricalcitol supplementation produced a reversible decline in eGFR.²⁰

There are some differences in the populations studied in the PENNY and the current study. In addition to racial differences, the mean age of the study participants in the current study was almost 18 years lower than that in PENNY study.²⁰

Our study has several strengths – it employed a directly observed therapy to ensure treatment compliance, used a rigorous, standardized methodology for FMD measurement, use of automated image detection and acquisition, and blinded randomized analysis that improved accuracy and reproducibility and removed bias. The limitation include its single center nature and exclusion of subjects with diabetes.

CONCLUSIONS

Correction of vitamin D deficiency by cholecalciferol supplementation improves CVD risk factor profile through positive impact on vascular function in non-diabetic subjects with early CKD, likely due to improvement in both endothelial and vascular smooth muscle cell functions. Short of clinical trials assessing impact of cholecalciferol supplementation on hard clinical endpoints, our observations provide strong evidence that cholecalciferol supplementation is safe and should be used for CV risk reduction in vitamin D deficient patients with CKD.

CONTRIBUTIONS

Concept and design: DB, VJ; Screening, enrolment and follow up of subjects: VK1, KLG; Performed pulse wave analysis: AKY; Performed flow mediated dilatation: AL; Analysed flow mediated dilatation data: MS; Performed laboratory experiments: AKY, VK2; Statistical analysis: LB; Wrote manuscript: VK1, AKY, VJ. All authors read and approved the manuscript.

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DISCLOSURES/CONFLICTS OF INTEREST

None.

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TABLES AND FIGURES LEGENDS

Table Legends

Table 1: Baseline characteristics of study subjects

Table 2: Baseline biochemical, circulating biomarkers and endothelial function in study subjects

Table 3: Change in endothelial function, biomarkers and biochemical parameters at 16 week follow-up

Figure Legends

Figure 1: Subject screening, enrolment and follow up (CONSORT 2010 flow diagram)

Figure 2: Forest plot showing effect of interaction between treatment and subgroup variable (gender, baseline vitamin D level tertiles, age group and use of renin angiotensin system inhibitors or statins) on primary outcome (change in endothelium dependent flow mediated dilatation)

TABLES

Table 1: Baseline characteristics of study subjects

Parameters	Placebo (n=59)	Cholecalciferol (n=58)
Gender (M/F)	40/19	41/17
Age (years)	45.20±11.61	43.17±11.79
Body mass index (kg/m ²)	23.45±2.91	23.57±2.67
SBP (mmHg)	127.88±15.67	128.48±14.43
DBP (mmHg)	82.33±10.21	83.38±10.09
Fasting blood sugar (mg/dl)	93.17±11.73	92.00±13.11
Duration of disease (months)	32.30±46.94	39.53±49.71
Number of subjects with proteinuria	30 (51)	31(53)
History of smoking	4 (7)	5 (9)
Hypertension	54 (92)	53 (91)
Family history of CAD	5 (8)	6 (10)
Family history of diabetes	17 (29)	14 (24)
Family history of kidney disease	7 (12)	8 (14)

Cause of CKD

Chronic Interstitial Nephritis	11(19)	10 (17)
Chronic Glomerulonephritis	5 (8)	6 (10)
Polycystic Kidney Disease	6 (10)	6 (10)
Unknown	30 (51)	27 (47)
Others	7 (12)	9 (15.51)

Medications

ACE inhibitors	23 (39)	24 (41)
ARB	16 (27)	18 (31)
Beta-blockers	17 (29)	20 (34)
Statins	19 (32)	27 (47)
Hypouricemic drugs	14 (24)	11 (19)
Calcitriol	1 (2)	3 (5)
Non-calcium based phosphate binders	1 (2)	2 (3)
Calcium based phosphate binders	7 (12)	6 (10)
Sodium bicarbonate	41 (69)	32 (55)
Calcium-channel blockers	38 (64)	36 (62)

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; CAD, coronary artery disease; CKD, chronic kidney disease; ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blockers.

Data presented as mean \pm standard deviation and number (percentage).

Table 2: Baseline biochemical, circulating biomarkers and endothelial function in study subjects

Parameter	Placebo (n=59)	Cholecalciferol (n=58)
Hemoglobin (g/dl)	12.02±1.94	11.97±1.69
eGFR (ml/min/1.73m ²)	34.63±12.25	35.77±12.37
Albumin (g/dl)	4.62±0.63	4.74±0.54
Calcium (mg/dl)	9.09±0.94	9.01±0.73
Inorganic Phosphorus (mg/dl)	4.03±1.39	3.65±0.91
Uric Acid (mg/dl)	7.66±2.37	8.01±2.32
Alkaline phosphatase (IU/L)	136.07±59.83	135.17±58.32
Total Cholesterol (mg/dl)	167.11±54.20	166.15±42.87
Triglyceride (mg/dl)	154.01±78.10	152.27±69.02
25(OH)D (ng/ml)	13.21±4.78	13.40±4.42
1,25 (OH) ₂ D(pg/ml)	20.11±12.54	19.10±10.69
iPTH (pg/ml) ^a	146 (102, 247)	139(84, 212)
E-selectin (ng/ml)	55.71±22.10	57.91±20.90
IL-6 (pg/ml) ^a	4.68 (2.59, 7.24)	4.44 (2.58, 7.10)

hs-CRP (mg/L) ^a	3.17 (0.94, 6.15)	2.2 (1.00, 4.45)
iFGF-23 (pg/ml) ^a	57.66 (44.48, 88.90)	57.88 (41.42, 69.04)
vWF (mU/ml) ^a	1050 (518, 1404)	1050 (704, 1362)
Brachial artery diameter (mm)	4.14±0.59	4.14±0.70
FMD (%)	7.85±2.35	7.65±2.25
NMD (%)	11.43±4.07	11.20±4.12
FMD/NMD ratio	0.69±0.47	0.80±0.57
PWV (m/sec)	7.98±1.74	7.98±1.63
AI (%) ^a	17 (7, 24)	22 (12, 26)

Abbreviations: eGFR, estimated glomerular filtration rate; 25(OH)D, 25 hydroxy vitamin D; 1,25 (OH)₂ D, 1,25 di-hydroxy vitamin D; iPTH, intact parathyroid hormone; IL-6, interleukin-6; hs-CRP, high sensitivity C-reactive protein; iFGF-23, intact fibroblast growth factor-23; vWF, Von Willebrand factor; FMD, endothelial dependent flow mediated dilatation; NMD, endothelial independent flow mediated dilatation; PWV, pulse wave velocity; AI, augmentation index.

SI conversion factors: to convert hemoglobin from g/dl to g/L, multiply by 10; albumin from g/dl to g/L, multiply by 10; calcium from mg/dl to mmol/L, multiply by 0.25; inorganic phosphorus from mg/dl to mmol/L, multiply by 0.323; serum urea from mg/dl to mmol/L, multiply by 0.357; uric acid from gm/dl to µmol/L, multiply by 59.48; triglyceride from mg/dL to mmol/L, multiply by 0.0113; cholesterol from mg/dl to mmol/L, multiply by 0.0259; 25(OH)

vitamin D from ng/ml to nmol/L, multiply by 2.5; 1,25 (OH)₂ vitamin D from pg/ml to pmol/L, multiply by 2.4 and intact parathyroid hormone pg/ml to pmol/L, multiply by 0.106.

Data presented as mean± standard deviation and median (25th, 75th percentile)

^a Compared using Mann-Whitney U test

Table 3: Change in endothelial function, biomarkers and biochemical parameters at 16 week follow-up

	Placebo (n=59)		Cholecalciferol (n=58)		Between group difference	
	Mean change (95% CI)	P value	Mean change (95% CI)	P value	Difference of Mean change (95% CI)	P value
Brachial artery diameter (mm)	0.10 (-0.01 to 0.21)	0.07	-0.08 (-0.20 to 0.04)	0.22	-0.18 (-0.52 to 0.07)	0.13
FMD (%)	-0.07 (-0.71 to 0.57)	0.83	5.42(4.44 to 6.39)	<0.001	5.49 (4.34 to 6.64)	<0.001
NMD (%)	-0.74 (-1.86 to 0.38)	0.19	2.11 (0.87 to 3.36)	0.001	2.85 (1.41 to 4.84)	<0.001
FMD/NMD ratio	0.02(-0.08 to 0.16)	0.54	0.27(0.10 to 0.44)	0.003	0.25 (0.05 to 0.46)	0.017
PWV (m/sec)	0.30 (-0.04 to 0.63)	0.09	-0.94 (-1.30 to -0.59)	<0.001	-1.24 (-2.16 to -0.74)	<0.001

AI (%) ^a	1.44 (-1.33 to 4.21)	0.19	0.16 (-2.50 to 2.81)	0.69	-1.28 (-5.11 to 2.48)	0.63
25(OH)D (ng/ml)	1.51 (-0.46 to 3.48)	0.13	24.91 (21.77 to 28.06)	<0.001	23.40 (19.76 to 27.06)	<0.001
1,25 (OH) ₂ D (pg/ml)	0.48 (-4.65 to 5.62)	0.85	15.46 (5.42 to 25.50)	0.003	14.98 (4.48 to 27.18)	0.007
i-PTH (pg/ml) ^a	47.36 (6.63 to 88.10)	0.050	-53.37 (-82.37 to - 24.36)	<0.001	-100.73 (-150.50 to - 50.95)	<0.001
E-selectin (ng/ml)	-2.43 (-6.78 to 1.92)	0.27	-4.71 (-8.89 to -5.25)	0.028	-2.28 (-8.26 to 3.70)	0.45
IL-6 (pg/ml) ^a	0.28 (-1.16 to 1.73)	0.17	-2.00(-2.96 to -0.83)	0.002	-2.28 (-3.96 to -0.38)	0.001
hs-CRP (mg/L) ^a	-0.41 (-1.07 to 2.45)	0.26	-0.25 (-0.99 to 0.43)	0.30	0.16 (-0.82 to 1.14)	0.94
iFGF-23 (pg/ml) ^a	-7.33 (-24.10 to 9.45)	0.27	-14.71 (-28.45 to -0.97)	0.33	-7.38 (-28.92 to 14.15)	0.96
vWF (mU/ml) ^a	74 (-136 to 284)	0.23	-140 (-311 to 30)	0.16	-214 (-483 to 54)	0.09
eGFR (min/ml/1.73m ²)	1.57(-0.93 to 4.01)	0.21	1.42 (-0.55 to 3.40)	0.15	-0.15 (-3.31 to 3.01)	0.92

Hemoglobin (g/dl)	-0.22 (-0.53 to 0.10)	0.18	-0.01 (-0.30 to 0.28)	0.94	0.21 (-0.22 to 0.63)	0.34
Uric Acid (mg/dl)	-0.51 (-0.67 to 0.08)	0.09	-0.60 (-1.12 to -0.03)	0.039	-0.09 (-0.89 to 0.72)	0.83
Calcium (mg/dl)	-0.48 (-0.76 to -0.19)	0.001	0.21 (-0.05 to 0.46)	0.11	0.69 (0.31 to 1.06)	0.001
Inorganic Phosphorus (mg/dl)	-0.30 (-0.67 to 0.08)	0.12	0.19 (-0.19 to 0.59)	0.31	0.49 (-0.37 to 1.03)	0.07
Serum alkaline phosphatase (IU/L)	9.40 (-2.08 to 20.89)	0.11	-10.85 (-20.70 to -1.01)	0.031	-20.25 (-35.14 to -5.38)	0.008
Total Cholesterol (mg/dl)	-3.03 (-13.14 to 7.09)	0.55	-8.15(-17.22 to 0.92)	0.08	-5.12 (-21.56 to 5.46)	0.29
Triglyceride (mg/dl)	0.56 (-20.77 to 21.89)	0.96	-8.38 (-24.41 to 7.65)	0.30	-8.94 (-35.13 to 17.23)	0.50

Abbreviations: eGFR, estimated glomerular filtration rate; 25(OH)D, 25 hydroxy vitamin D; 1,25 (OH)₂ D, 1,25 di-hydroxy vitamin D; iPTH, intact parathyroid hormone; IL-6, interleukin-6; hs-CRP, high sensitivity C-reactive protein; iFGF-23, intact fibroblast growth factor-23; vWF, Von Willebrand factor; FMD, endothelial dependent flow mediated dilatation; NMD, endothelial independent flow mediated dilatation; PWV, pulse wave velocity; AI, augmentation index.

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^aCompared using Willcoxon-signed rank test within group and Mann-Whitney U test between group

Figure 1

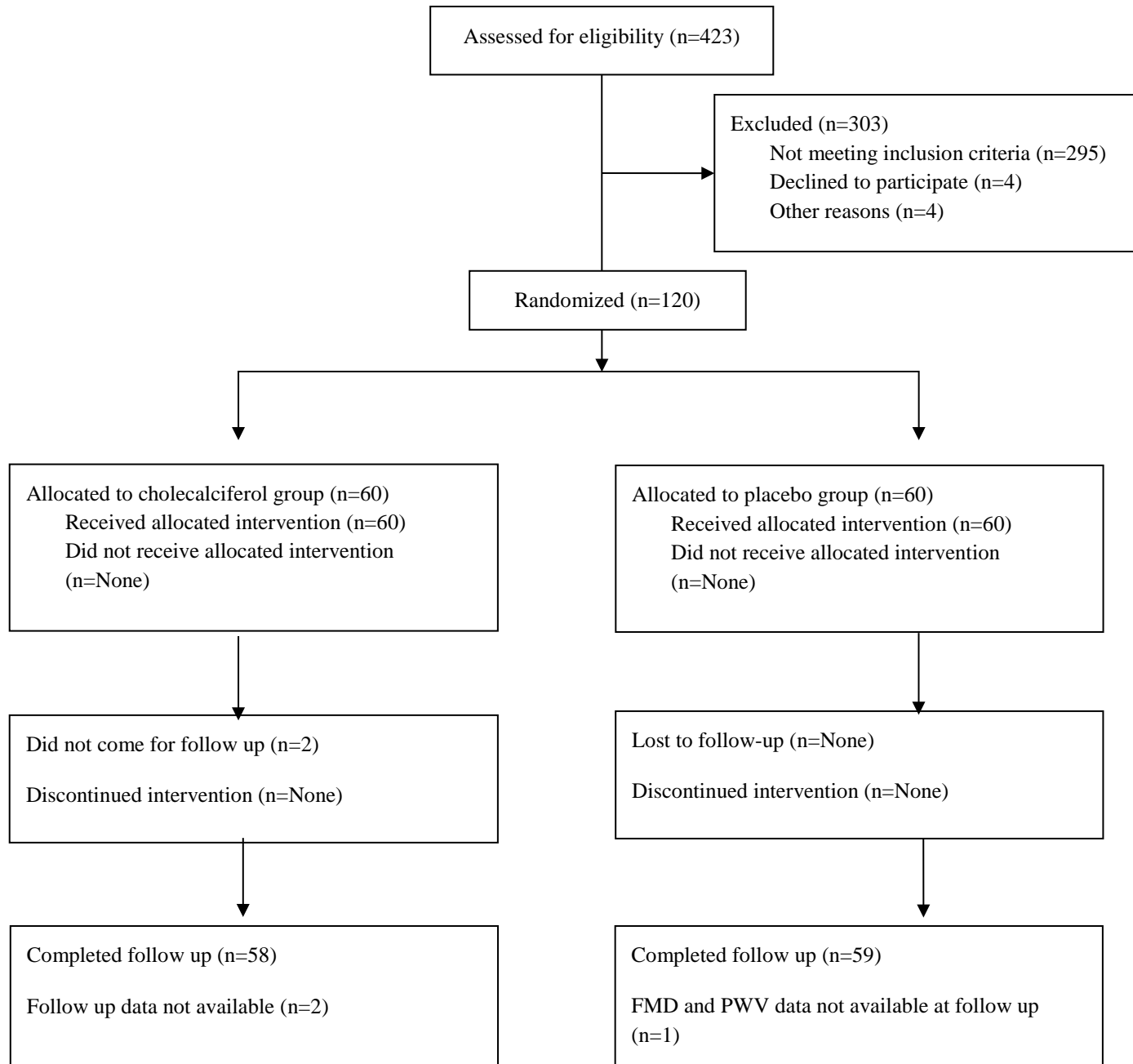
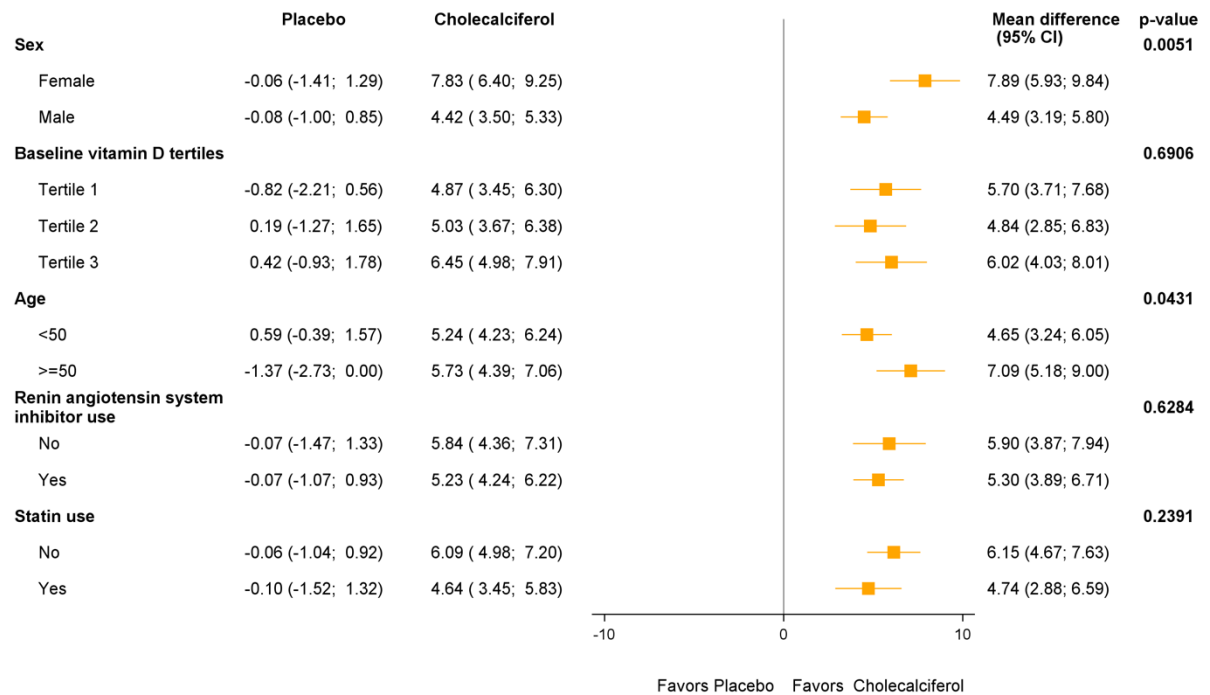


Figure 2



p-value is from test statistics for interaction between treatment and subgroup variable. Data presented as mean change (95% confidence interval)

SUPPLEMENTARY FILES

Table S1: FMD and NMD at baseline and 16 week visits

Table S2: Adverse events recorded during the study (number of subjects)

Supplementary figure S1: Comparison between selected parameters at 16 weeks and change over 16 weeks in either group

SUPPLEMENTARY TABLES

Table S1: FMD and NMD at baseline and 16 week visits

Parameter	Placebo (n=59)	Cholecalciferol (n=58)	P value
At Baseline			
FMD (%)	7.85±2.35	7.65±2.25	0.65
NMD (%)	11.43±4.07	11.20±3.91	0.59
Shear stress (m/sec)	189.27±84.37	186.44±74.14	0.85
At 16 weeks			
FMD (%)	7.78±2.90	13.07±3.96	<0.001
NMD (%)	10.69±3.44	13.31±3.97	<0.001
Shear stress (m/sec)	180.50±71.38	184.00±74.94	0.80
Proportional increase in FMD (%) ^a	2.78 (- 16.50,13.58)	62.95 (34.96- 96.65)	<0.001
Proportional increase in NMD (%) ^a	-4.38 (-31.61, 12.38)	12.40 (-5.54, 51.39)	<0.001

Abbreviations: FMD, endothelium dependent flow mediated dilatation; NMD, endothelium independent nitroglycerine mediated dilatation.

Data presented as mean± standard deviation and median (25th, 75th percentile).

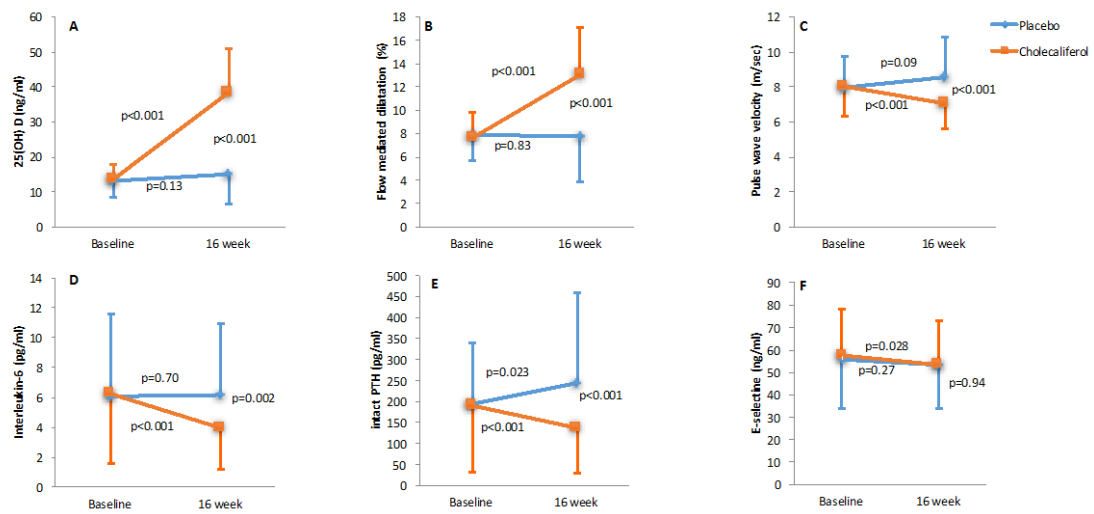
^aCompared using Mann-Whitney U test

Table S2: Adverse events recorded during the study (number of subjects)

Adverse events	Placebo	Cholecalciferol
Bodyaches	4	1
Dyspepsia	1	2
Mild lower limb edema	1	2
Upper respiratory tract infection	1	2
Urinary tract infection	0	1
Dengue	1	0
Lower limb cellulitis	1	0
Excessive hair growth	0	1
Hypercalcemia (serum calcium >10.5 mg/dl)	1	0

SUPPLEMENTARY FIGURE

Figure S1



PTH: parathyroid hormone