Clinical Trial

The Effect of Vitamin D Supplementation on Bone Metabolic Markers in Chronic Kidney Disease[†]

Running head: Vitamin D supplementation and bone metabolic markers in CKD

Ashok Kumar Yadav[1]*, Vivek Kumar[1]*, Vinod Kumar[1], Debasish Banerjee[2], Krishan Lal Gupta[1], Vivekanand Jha[1,3,4]

[1]Department of Nephrology, Postgraduate Institute of Medical Education and Research, Chandigarh, India
[2]Renal and Transplantation Unit, St George's University Hospitals NHS Foundation Trust, Molecular and Clinical Sciences Research Institute, St Georges, University of London, United Kingdom
[3]The George Institute for Global Health, New Delhi, India
[4]University of Oxford, Oxford, England, United Kingdom

*Equal contribution as first author

Correspondence to: Prof Vivekanand Jha, George Institute for Global Health, 311-312, Third Floor, Elegance Tower,Plot No. 8, Jasola District Centre, New Delhi 110025 INDIA
Email: vjha@pginephro.org

ABSTRACT

Use of active forms of vitamin D is advocated in CKD patients for treatment of mineral bone disease because of the presumption that native forms of vitamin D would not undergo significant activation to calcitriol, the most active biological form of vitamin D. We present secondary analysis looking at bone turnover in subjects who completed the randomized, double blind, placebo controlled trial investigating the effect of cholecalciferol supplementation on vascular function in non-diabetic CKD stage G3-4 and vitamin D ≤20ng/ml [CTRI/2013/05/003648]. Patients 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. Of the 120 subjects enrolled, 58 in the cholecalciferol group and 59 in the placebo group completed the study. At 16 weeks, the serum 25(OH)D and 1,25(OH)2D levels increased in the cholecalciferol group but not in the placebo group [between-group difference in mean change: 23.40 ng/ml; 95% CI: 19.76 to 27.06; p<0.001 and 14.98 pg/ml ,95% CI: 4.48 to 27.18, p=0.007, respectively]. Intact parathormone (iPTH) decreased in the cholecalciferol group [between-group difference in mean change -100.73 pg/ml (95% CI: -150.50 to -50.95, p<0.001). Serum total and bone-specific alkaline phosphatase (SAP, BAP) and serum C-terminal cross-linked collagen type I telopeptides (CTX-1) were significantly reduced in cholecalciferol group (between group difference for change in mean: -20.25 U/L (95% CI: -35.14 to-5.38, p=0.008 for SAP; -12.54, 95%CI: -22.09 to -2.98, p=0.013 for BAP and -0.21, 95%CI: -0.38 to -0.05, p=0.05 for CTX-1). Correlation analysis showed significant correlation of Δ 25(OH)D with Δ iPTH (r=-0.409, p<0.0001), Δ 1,25(OH)2D (r=0.305, p=0.001), Δ SAP (r=-0.301, p=0.002), ΔBAP (r=-0.264, p=0.004), and ΔCTX-1 (r=-0.210, p=0.0230). Cholecalciferol supplementation corrects vitamin D deficiency and is effective in lowering serum intact parathyroid hormone and bone turnover markers in early stages of CKD. This article is protected by copyright. All rights reserved

Keywords

Chronic kidney disease, cholecalciferol, hyperparathyroidism, Fibroblast growth factor 23, bone turnover.

BACKGROUND

Mineral and bone disorders (MBD) are common, and start early in the course of chronic kidney disease (CKD).[1] Current understanding indicates that vitamin D, calcium, parathyroid hormone (PTH), inorganic phosphate and fibroblast growth factor 23 (FGF23) are key players in CKD-MBD.[2] Clinical manifestations appear relatively late, but biochemical abnormalities, such as elevations in serum phosphate, FGF23 and parathyroid hormone levels have been noted in 3%, 50% and 22% of subjects with eGFR 50-59 ml/min/1.73m^2.[3]

Abnormality in vitamin D production is central to the genesis of CKD-MBD. Reduced renal 1-α hydroxylase activity lowers the levels of activated vitamin D3, and causes an increase in PTH secretion. Vitamin D deficiency stimulates the secretion of FGF23 by osteocytes, which further contributes to hyperparathyroidism.[4,5] The discovery of 1-α hydroxylase activity in many tissues, including the parathyroid gland suggests that correction of vitamin D deficiency might ameliorate the secondary hyperparathyroidism of CKD by improving substrate availability, at least in early stages of the disease. Cross-sectional studies have shown an association between circulating vitamin D levels and markers of bone turnover in CKD.[6] However, whether correction of vitamin D deficiency will lead to improvement in CKD-MBD has not been tested in interventional studies.

In a pre-specified secondary analysis of randomised double-blind placebo-controlled trial of cholecalciferol supplementation in non-diabetic vitamin D deficient subjects with stage 3-4 CKD, we investigated the effect of oral cholecalciferol supplementation on markers of mineral metabolism which included 1,25(OH)$_2$D, FGF23, PTH, serum C-terminal cross-linked collagen type I telopeptides (CTX-1), total and bone-specific alkaline phosphatase (BAP).

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METHODS

Study subjects and biomarker evaluation

This single centre, parallel arm, randomized, double blind, placebo-controlled trial was done at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India. The study was approved by Institute Ethics Committee and all subjects provided written informed consent. The detailed study protocol and subject enrolment process has been described earlier.[7] Briefly, 120 stable non-diabetic, CKD stage G3-4 subjects of either sex between the ages of 18 and 70 years with serum 25(OH)D levels ≤20 ng/ml were randomised in a 1:1 allocation ratio to receive two directly supervised doses of either 300,000 IU of cholecalciferol or matching placebo at baseline and 8 weeks later. Randomization was done by computer generated Bernoulli random number table, and the investigators as well as the patients were blinded.

Serum creatinine, calcium, inorganic phosphorus, lipid profile, uric acid, and haemoglobin were measured at baseline and 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).[8,9] 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], BAP [MicroVue^{TM} BAP EIA, Quidel Corporation San Diego USA] and CTX-1 [Serum CrossLaps® (CTX-1) ELISA, IDS, UK) were analysed in all samples.

Outcomes

We investigated changes in levels of serum levels of 1,25$(OH)_2$D, intact-PTH, intact-FGF23, inorganic phosphorus, calcium and SAP, BAP and CTX-1.

Statistical analysis

Data are presented as mean ± standard deviation, mean (95% confidence interval) and median (interquartile range) as appropriate. 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. Correlation analysis was performed using Spearman's rank correlation. Subgroup analysis based on gender, baseline vitamin D tertiles and age (≤50 years and >50 years) was performed to see any effect on change in iPTH within subgroups. Interaction between intervention and subgroup variables was examined using general linear model. 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).

RESULTS

Baseline characteristics of the study subjects are shown in Table 1. The demographic details, causes of CKD and use of medications were similar in both the groups. Biochemical parameters and circulating biomarker levels did not differ between the groups at baseline (Table 2).

Change in biochemical parameters and circulating biomarkers

At 16 weeks, the serum 25(OH)D levels increased in the cholecalciferol group (mean change: +24.91 ng/ml; 95% CI: 21.77 to 28.06; p<0.001; table 3) but not in the placebo group (+1.51. ng/ml; 95% CI: (-0.46 to 3.48; p=0.13; table 3). The difference in change in 25(OH)D levels between the two groups was significant (p<0.001, Table 3, 4). Similarly, serum $1,25(OH)_2D$ levels increased in the cholecalciferol (15.46 pg/ml; 95% CI: 5.42 to 25.50, p=0.003) but not in placebo group (0.48 pg/ml; 95% CI: -4.65 to 5.62, p=0.85), with significant difference between the group for change over 16 weeks (p=0.007).

Serum levels of iPTH showed a significant decline in the cholecalciferol group at 16 weeks whereas in placebo group there was a rise. This was accompanied by a significant decrease in total SAP, BAP and CTX-1 levels in cholecalciferol group, whereas the levels remained unchanged in the placebo treated subjects. The between group difference in change was significant for all these markers (Table 3,4).

Though serum level of iFGF23 did show a decreasing trend in the cholecalciferol group, it did not reach statistical significance. Change in iFGF23 in the placebo group, and between group difference for change in iFGF23 were not significant. A numeric increase was noted in serum calcium in the cholecalciferol group, whereas it decreased significantly in placebo group, leading to a significant between-group difference in mean change. Serum uric acid decreased in the cholecalciferol-treated subjects but not in placebo group. Serum inorganic phosphorus (Pi), serum creatinine, eGFR and hemoglobin levels did not change significantly in either group (Table 3,4).

Correlation analysis for biomarkers of mineral metabolism

We examined the correlation of change (Δ) in 25 (OH) D levels at baseline and at 16 weeks with that in the other markers, and found a significant correlation of Δ 25(OH)D with Δ iPTH (r=-0.403, p<0.0001), Δ 1,25(OH)$_2$D (r=0.305, p=0.001), Δ SAP (r=-0.301, p=0.002), ΔBAP (r=-0.264, p=0.004), ΔCTX-1 (r=-0.210, p=0.0230), Δ Pi (r=0.220, p=0.019), and Δ Ca (r=0.199, p=0.05). ΔiFGF23 correlated with ΔiPTH (r=0.290, p=0.002) but not with Δ 25(OH)D (r=-0.121, p=0.195).

In subgroup analyses, the change in iPTH remained significant in males, lower tertiles (tertile 1 and 2) of baseline vitamin D levels and groups based on age ($\leq$50 years and >50 years) with no significant heterogeneity of treatment effect on ΔiPTH across these subgroups (table 5).

DISCUSSION

This study provides strong evidence that correction of native vitamin D deficiency with cholecalciferol supplementation efficiently achieves sufficient level of 25(OH)D in pre-dialysis CKD subjects and has a favourable effect on several biochemical parameters of CKD-MBD, including suppression of secondary hypoparathyroidism, change in bone turnover markers and favourable trend in FGF-23 levels. Change in serum 25(OH)D level significantly correlated with the change in markers of mineral metabolism, indicating that vitamin D deficiency is a potentially modifiable risk factor for bone mineral disease in subjects with stage G3-4 CKD.

Vitamin D deficiency has been shown to be associated with hyperparathyroidism and increased bone turnover in subjects with CKD, as shown by elevation in CTX-1 and BAP.[6] An association of SAP with vitamin D deficiency has been documented in various settings.[10-12]

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Malachi et al reported high level of SAP in vitamin D deficient elderly individuals and decline with seasonal rise in vitamin D level. [13] Other observational studies and randomised trials have investigated the effect of Vitamin D supplementation in healthy adults and children. Most showed a significant decline in PTH, but the effect on bone formation and resorption markers was inconsistent. [14-19] Chandra et al randomized 34 subjects with stage 3-4 CKD and 25(OH) D level <30 ng/ml to receive weekly 50,000 IU cholecalciferol or placebo for 12 week. There was a decrease in the week 12 iPTH levels, but there was no difference in the levels of bone turnover markers. [20]

Despite the efficacy of cholecalciferol supplementation in reducing iPTH levels in subjects with pre-dialysis CKD,[21,22] recent studies have focussed on activated vitamin D analogues.[23] The findings of the present study show that a sufficient level of circulating 25(OH) D in subjects with CKD is important to restrict PTH secretion/synthesis and reduce bone turnover, and could be particularly relevant in areas with widespread vitamin D deficiency as a cheap alternative.

PTH suppression can be mediated by several mechanisms. 25(OH)D is substrate for renal 1-α hydoxylase, which produces 1,25(OH)$_2$D from circulating 25(OH)D, that binds to vitamin D receptor on parathyroid cells, to suppress the secretion of PTH.[24] This was supported by the finding that vitamin D supplementation led to an increase in levels of circulating 1,25 (OH)$_2$ D$_3$. Parathyroid gland cells have also been shown to express 1-α hydoxylase, which can suppress PTH by local production of 1,25 (OH)$_2$ D$_3$. Finally, an increase in calcium level can supress the PTH secretion, but whether the level can go down to the normal remains a matter of investigation. Though between group difference in serum uric acid levels was not significant, decrease in serum uric acid levels was noted in the cholecalciferol group. PTH

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might modulate serum uric acid levels, as suggested in a recent report where elevated PTH was shown to impair uric acid excretion from kidneys and gut in CKD animals. [25]

Increased FGF23 can inhibit the PTH expression, mediated by Klotho-FGFR1c activity in CKD subjects.[4] Elevated FGF23 levels are also associated with decrease in $1,25(OH)_2D$ because of their suppressive effect on 1-α hydroxylase.[22] In this context, the declining trend in FGF23 with vitamin D supplementation, and the positive correlation of change in FGF23 with change in iPTH are of interest. Some studies have reported a rise in FGF23 level after supplementation of cholecalciferol [22] and paricalcitol.[23] In our study, fall in PTH levels and elevated 1.25 (OH) D would have had competing effects on FGF23. [26] The interactions of FGF23 with iPTH, $1,25(OH)_2D$ and 25(OH) D is complicated and needs further investigation.

Vitamin D deficiency is almost universal in CKD population. The recently updated KDIGO CKD-MBD Guidelines[27] make an ungraded recommendation to correct vitamin D deficiency in CKD subjects. Also, the exact protocol for correction is not well-defined. We used two doses of 300,000 IU 8 week apart, which effectively replenished the 25(OH)D levels accompanied by increase in active vitamin D and reduction in iPTH level and markers of bone turnover and no events of hypercalcemia and hyperphosphatemia. However, this strategy needs to be monitored for its long term impact.

There are some limitations of our study which includes single centre design, exclusion of diabetic subjects, absence of bone biopsy, lack of assessment of dietary intake and urinary excretion of calcium and phosphate and a short duration of intervention and follow-up. The strength of this study is that it was randomised, double-blind, placebo-controlled study with adequate sample size.

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In conclusion, correction of vitamin D deficiency with cholecalciferol supplementation suppress secondary hyperparathyroidism and favourably change biochemical markers of mineral metabolism in subjects with stage 3-4 CKD.

Disclosures

None

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Author's contributions

Research idea and study design: Vivek K, VJ; data acquisition: AKY, Vinod K; data analysis/interpretation: Vivek K, AKY, Vinod K, VJ; statistical analysis: Vivek K, AKY; supervision or mentorship: DB, KLG, VJ.

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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
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)
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 pressur; 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± standard deviation and number (percentage).

Table 2: Key laboratory findings and levels of circulating biomarkers in study subjects

Parameter	Placebo (n=59)	Cholecalciferol (n=58)
Hemoglobin (mmol/L)	7.46±1.20	7.43±1.05
eGFR (min/ml/1.73m^2)	34.63±12.25	35.77±12.37
Albumin (g/L)	46.20±6.30	47.40±5.40
Calcium (mmol/L)	2.27±0.23	2.25±0.13
Inorganic Phosphorus (mmol/L)	1.30±0.45	1.18±0.29
Uric Acid (µmol/L)	455.62±140.97	476.43±138.00
25(OH)D (nmol/L)	32.97±11.93	33.45±4.42
1,25 (OH)$_2$ D(pmol/L)	50.19±31.30	47.67±27.53
iPTH (pg/ml)[*]	146 (102, 247)	139(84, 212)
iFGF-23 (pg/ml) [*]	57.66 (44.48, 88.90)	57.88 (41.42, 69.04)
SAP (U/L)	136.07±59.83	135.17±58.32
BAP (U/L)*	40.06 (29.34, 55.89)	37.59 (27.49, 52.56)
CTX-1 (ng/ml)*	1.44 (1.00, 2.33)	1.45 (0.82, 2.23)

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; iFGF-23, intact fibroblast growth factor-23; SAP, total serum alkaline phosphatase; BAP, bone specific alkaline phosphatase; CTX-I, C-terminal telopeptides of Type 1 collagen

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

[*] Compared using Mann-Whitney U test

Table 3: Change in biochemical parameters and markers of bone turnover between baseline and 16 weeks

	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
25(OH)D (nmol/L)	3.77 (-1.15 to 8.69)	0.13	62.18 (54.69 to 70.04)	**<0.001**	58.41 (49.32 to 67.54)	**<0.001**
1,25 (OH)$_2$ D (pmol/L)	1.20 (-11.61 to 14.03)	0.85	38.59 (13.53 to 63.65)	**0.003**	37.39 (11.18 to 67.84)	**0.007**
i-PTH (pg/ml) [*]	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**
iFGF-23 (pg/ml) [*]	-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
SAP (U/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**
BAP (U/L)*	-4.67 (-10.70 to 1.37)	0.068	-17.20 (-24.74 to -9.66)	**<0.001**	-12.54 (-22.09 to -2.98)	**0.013**

CTX-I (ng/ml)*	-0.09 (-0.22 to 0.02)	0.132	-0.31 (-0.42 to -0.20)	**<0.001**	-0.21 (-0.38 to -0.05)	**0.05**
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 (mmol/L)	-0.14 (-0.33 to 0.06)	0.18	-0.01 (-0.19 to 0.17)	0.94	0.13 (-0.14 to 0.39)	0.34
Uric Acid (mmol/L)	-30.33 (-39.85 to 4.76)	0.09	-35.69 (-66.12 to -1.78)	**0.039**	-5.36 (-52.94 to 42.82)	0.83
Calcium (mmol/L)	-0.12 (-0.19 to -0.05)	**0.001**	0.05 (-0.01 to 0.11)	0.11	0.17 (0.08 to 0.26)	**0.001**
Inorganic Phosphorus (mmol/L)	-0.10 (-0.22 to 0.03)	0.12	0.06 (-0.06 to 0.19)	0.31	0.16 (-0.12 to 0.33)	0.07

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; SAP, total serum alkaline phosphatase; BAP; bone specific alkaline phosphatase; CTX-I, C-terminal telopeptides of Type 1 collagen

*Compared using Willcoxon-signed rank test within group and Mann-Whitney U test between groups

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Table 4: Percent change at 16 week from baseline in circulating biomarkers in study subjects

Parameter	Placebo (n=59)	Cholecalciferol (n=58)	P value
25(OH)D	3.79 (-25.80, 34.89)	197.70 (116.18, 290.55)	<0.0001
1,25(OH)$_2$ D	-6.48 (-38.16, 49.66)	38.90 (-3.47, 158.03)	0.002
iPTH	16.66 (-18.80, 93.56)	-25.63 (-40.09, 1.87)	<0.0001
iFGF-23	-2.12 (-34.91, 49.66)	-1.22 (-36.83, 17.09)	0.892
SAP	0.00 (-4.46, 20.77)	-10.44 (-22.04, 1.81)	0.001
BAP	-5.36 (-38.20, 34.80)	-29.96 (-61.77, 14.29)	0.025
CTX-1	-2.92 (-27.33, 16.73)	-18.02 (-41.02, 0.00)	0.008
Calcium	-4.39 (-12.20, 3.33)	1.66 (-3.51, 9.06)	0.001
Inorganic Phosphorus	-9.02 (-23.84, 13.86)	0.00 (-11.72, 21.87)	0.066

Abbreviations: 25(OH)D, 25 hydroxy vitamin D; 1,25 (OH)$_2$ D, 1,25 di-hydroxy vitamin D; iPTH, intact parathyroid hormone; iFGF-23, intact fibroblast growth factor-23; SAP, total serum alkaline phosphatase; BAP, bone specific alkaline phosphatase; CTX-I, C-terminal telopeptides of Type 1 collagen

Data presented as median (25th, 75th percentile)

p value obtained using Mann-Whitney U test

Table 5: Change in iPTH at 16 weeks based on gender, baseline vitamin D level tertiles and age group

	Placebo	Cholecalciferol	p value
Gender			
Female	N=19	N=17	0.402
	22.89 (-81.23 to 127.00)	-48.57(-110.38 to 13.25)	
Male	N=40	N=41	
	58.70 (19.71 to 97.69)	-55.36(-89.26 to -21.44)	
Baseline vitamin D level tertiles			
Tertile 1	N=21	N=19	0.078
(4.46-10.08 ng/ml)	71.21 (-13.56 to 156.00)	-74.08 (-112.02 to -32.65)	
Tertile 2	N=18	N=21	
(10.09-16.67 ng/ml)	85.07 (0.94 to 169.21)	-57.48 (-90.26 to -24.71)	
Tertile 3	N=20	N=18	
(16.68-19.83 ng/ml)	-8.82 (-52.30 to 34.66)	-26.69 (-66.63 to 13.22)	
Age group			
≤50 years	N=40	N=40	0.435
	14.19 (-30.88 to 59.36)	-72.34 (-112.02 to -32.65)	
>50 years	N=19	N=18	
	118.94 (37.90 to 199.98)	-11.20 (-37.27 to 14.87)	

Data presented as mean (95% confidence interval), *p value for test of interaction between treatment and subgroup variables

by general linear model.