Supplementary Material for:

Effects of Vitamin D_2 Supplementation on Vitamin D_3 Metabolism in Health and Chronic Kidney Disease

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Supplementary Methods

Exclusion criteria

Exclusion criteria included rapidly advancing renal failure, treatment with a vitamin D receptor agonist or phenytoin, current hospitalization or within the previous 6 weeks, pregnancy, breastfeeding, anemia (hematocrit <27%), serum calcium >10.2 mg/dL, history of parathyroid disease, 1+ or greater dipstick proteinuria (for healthy subjects only), history of gastrointestinal diseases that could impede absorptive ability, liver disease (ALT or AST > 100U/L) or cholestasis (direct bilirubin >1.0 mg/dL, because of the limitation of fat soluble vitamin absorption in this setting), and renal replacement therapy.

Estimation of circulating bioavailable 25-hydroxyvitamin D concentrations

Circulating bioavailable 25-hydroxyvitamin D concentrations were estimated from plasma concentrations of total 25-hydroxyvitamin D, vitamin D binding protein, and albumin and vitamin D binding protein isoform using published methods, described here.

Abbreviations and Definitions:

25(OH)D = 25-hydroxyvitamin D

$[D_{total}]$ = plasma 25(OH)D concentration= $[25(OH)D_2 + 25(OH)D_3]$

$[D_{free}]$ = free plasma 25(OH)D concentration

$[D_{bio}]$ = bioavailable D concentration= $[D_{free}] + [D_{Alb}]$

[Alb] = Albumin

$[D_{Alb}]$ = concentration of albumin-bound 25(OH)D

[VDBP] = vitamin D binding protein concentration

$[D_{DBP}]$ = concentration of D binding protein –bound to 25(OH)D

$[D_{free}DBP]$ = concentration of unbound DBP = total VDBP - $[D_{DBP}]$

K_{alb} = affinity constant between 25(OH)D and albumin= $6x10^5\ M^{-1}$

K_{DBP} = affinity constant between 25(OH)D and DBP with haplotype specific affinity constants as follows:

if haplotype was homozygous Gc1F, $K_{DBP} = 6.8 \times 10^8\ M^{-1}$

if haplotype was homozygous Gc1F, $K_{DBP} = 11.2 \times 10^8\ M^{-1}$

if haplotype was homozygous Gc2, $K_{DBP} = 3.6 \times 10^8\ M^{-1}$

if haplotype was heterozygous Gc2/Gc1F, $K_{DBP} = 7.4 \times 10^8\ M^{-1}$

if haplotype was heterozygous Gc2/Gc1S, K_{DBP} = 4.8 x 10^8 M^{-1}

The affinity constants were determined in a prior study by Arnaud and Constans [1]. The binding coefficients for heterozygous haplotypes were the mean of the 2 binding coefficients of the specific DBP carriers [2].

Known equations adapted from Powe, et al [3].

1. $[D_{Alb}] = K_{alb} \times [Alb] \times [D_{free}]$
2. $[D_{DBP}] = [D_{total}] - [D_{Alb}] - [D_{free}]$
3. $[D_{free}] = [D_{DBP}] \div K_{DBP} \div [D_{free}DBP]$
4. $[D_{free}DBP]$ = total VDBP - $[D_{DBP}]$

Therefore, using equations 3 and 4:

$[D_{free}] = [D_{DBP}] \div K_{DBP} \div ([VDBP] - [D_{DBP}])$

and using equations 1 and 2:

$[D_{DBP}] = [D_{total}] - (K_{alb} \times [Alb] + 1) \times [D_{free}]$

and

$[D_{free}] = ([D_{total}] - (K_{alb} \times [Alb] + 1) \times [D_{free}]) \div K_{DBP} ([VDBP] - ([D_{total}] - (K_{alb} \times [Alb] + 1) \times [D_{free}]))$

Further simplified into a polynomial equation (second-degree): $ax^2 + bx + c$

Where

$x = [D_{free}]$

$a = K_{DBP} \times K_{alb} \times [Alb] + K_{DBP}$

$b = K_{DBP} \times [VDBP] - K_{DBP}[D_{total}] + K_{alb} \times [Alb] + 1$

$c = -[D_{total}]$

Therefore: $[D_{free}] = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$

Bioavailable D can then be calculated:

$[D_{bio}] = [D_{free}] + [D_{Alb}] = (K_{alb} \times [Alb] + 1) \times [D_{free}]$

References for calculations:

1. Arnaud J, Constans J. Affinity differences for vitamin D metabolites associated with the genetic isoforms of the human serum carrier protein (DBP). *Human genetics.* Sep 1993;92(2):183-188.
2. Johnsen MS, Grimnes G, Figenschau Y, Torjesen PA, Almas B, Jorde R. Serum free and bio-available 25-hydroxyvitamin D correlate better with bone density than serum total 25-hydroxyvitamin D. *Scand J Clin Lab Invest.* Apr 2014;74(3):177-183.
3. Powe CE, Ricciardi C, Berg AH, et al. Vitamin D-binding protein modifies the vitamin D-bone mineral density relationship. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research.* Jul 2011;26(7):1609-1616.

Supplementary Table S1: Baseline characteristics of participants by screening eGFR (based on local creatinine measurement and the MDRD formula) and baseline eGFR (based on central creatinine and cystatin C measurement and the CKD-EPI formula), identifying 16 participants with discordant eGFR classification.

Characteristic	Estimated GFR		
	Screening and baseline eGFR <60 mL/min/1.73m^2 (N=24)	**Discordant screening and baseline eGFR (N=16*)**	**Screening and baseline eGFR ≥60 mL/min/1.73m2 (N=29)**
Age	61.8±12.9	53.6±15.6	51.8±13.6
Sex			
Male	18 (75)	8 (50)	15 (51.7)
Female	6 (25)	8 (50)	14 (48.3)
Race/ethnicity			
White	13 (54.2)	12 (75.0)	14 (48.3)
Black	8 (33.3)	4 (25.0)	6 (20.7)
Hispanic	3 (12.5)	0 (0.0)	7 (24.1)
Asian	0 (0.0)	0 (0.0)	2 (6.9)
Baseline creatinine (mg/dL)	2.0±0.9	1.1±0.3	0.8±0.2
Baseline estimated GFR (mL/min/1.73m^2)	39.3±12.6	82.0±18.9	135.6±44.6
Baseline estimated GFR category			
≥60 mL/min/1.73m^2	0 (0.0)	15 (93.8)	29 (100.0)
45-59 mL/min/1.73m^2	8 (33.3)	1 (6.3)	0 (0.0)
30-44 mL/min/1.73m^2	11 (45.8)	0 (0.0)	0 (0.0)
15-29 mL/min/1.73m^2	4 (16.7)	0 (0.0)	0 (0.0)
<15 mL/min/1.73m^2	1 (4.2)	0 (0.0)	0 (0.0)
Screening creatinine	2.1±0.8	1.5±0.3	1.0±0.2
Screening eGFR	36.5±10.3	48.9±8.7	77.4±12.3
Screening eGFR category			
≥60 mL/min/1.73m^2	0 (0.0)	1(6.3)	29 (100)

45-59 mL/min/1.73m^2	6 (25.0)	11 (68.8)	0 (0.0)
30-44 mL/min/1.73m^2	14 (58.3)	4 (25.0)	0 (0.0)
15-29 mL/min/1.73m^2	3 (12.5)	0 (0.0)	0 (0.0)
<15 mL/min/1.73m^2	1 (4.2)	0 (0.0)	0 (0.0)

Cell contents are presented as N (%) or mean (± standard deviations). Screening eGFR was based on local creatinine measurements and calculated using the MDRD formula, and baseline eGFR was based on central creatinine and cystatin C measurement and calculated using the CKD-EPI formula. (*) The discordant group includes 15 participants with screening GFR <60 and baseline eGFR ≥60 mL/min/1.73m^2 and 1 participant with screening GFR ≥60 and baseline eGFR <60 mL/min/1.73m^2.

Supplementary Table S2: Mineral Metabolism Biomarkers by CKD status, before and after treatment, excluding 16 participants with discordant screening and baseline eGFR values.

Biomarker	CKD (eGFR <60 mL/min/1.73m^2)				Non-CKD (eGFR ≥60 mL/ min/1.73m^2)				Difference in Change by CKD status	
	Baseline	Post-treatment	Change (95% CI)	p-value	Baseline	Post-treatment	Change (95% CI)	p-value	Difference in change (95% CI)	p-value interaction
25(OH)D total (ng/mL)	19.7 (1.1)	40.7 (1.9)	21.0 (16.8, 25.3)	<0.001	18.8 (1.7)	37.6 (1.7)	18.8 (14.8,22.8)	<0.001	3.9 (-2.6,10.4)	0.24
25(OH)D_3 (ng/mL)	17.6 (1.1)	10.0 (1.1)	-7.6 (-9.3,-5.9)	<0.001	16.2 (1.0)	8.0 (1.0)	-8.2 (-9.7,-6.6)	<0.001	0.5 (-1.9,2.8)	0.70
25(OH)D_2 (ng/mL)	2.1 (1.7)	30.9 (1.8)	28.8 (24.5,33.1)	<0.001	2.6 (1.6)	29.5 (1.6)	26.9 (23.0,30.9)	<0.001	3.7 (-2.6,10.0)	0.25
1,25(OH)$_2$D total (pg/mL)	30.9 (2.7)	34.4 (2.8)	3.5 (-1.9,8.9)	0.21	49.9 (2.5)	49.6 (2.6)	-0.2 (-5.3,4.8)	0.92	2.8 (-4.6,10.1)	0.46
1,25(OH)$_2D_3$ (pg/mL)	28.7 (2.4)	10.43 (2.5)	-18.4 (-23.9,-12.8)	<0.001	47.3 (2.2)	15.4 (2.3)	-31.9 (-37.0,-26.8)	<0.001	13.0 (5.2,20.8)	0.001
1,25(OH)$_2D_2$ (pg/mL)	2.2 (1.7)	24.1 (1.8)	21.9 (17.3,26.6)	<0.001	2.6 (1.6)	34.2 (1.6)	31.6 (27.3,35.9)	<0.001	-10.2 (-16.5,-3.8)	0.002
24,25 (OH)$_2D_3$ (ng/mL)	1.2 (0.2)	1.0 (0.2)	-0.3 (-0.5,-0.0)	0.06	2.1 (0.2)	1.5 (0.2)	-0.5 (-0.8,-0.3)	<0.001	0.2 (-0.1,0.6)	0.21
PTH (pg/mL)	116.6 (10.6)	101.1 (10.6)	-15.6 (-28.0,-3.1)	0.01	57.6 (9.8)	49.4 (9.8)	-8.2 (-19.8,3.3)	0.16	-6.3 (-23.9,11.4)	0.49
FGF-23 (pg/mL)	86.9 (6.6)	89.6 (6.6)	2.7 (-5.8,11.2)	0.54	41.2 (6.1)	44.3 (6.1)	3.0 (-4.8,10.9)	0.45	0.2 (-11.7,12.0)	0.98
Calcium (mg/dL)	9.0 (0.2)	8.9 (0.2)	-0.1 (-0.4,0.1)	0.30	9.0 (0.2)	9.0 (0.2)	0.0 (-0.2,0.3)	0.83	-0.2 (-0.6,0.2)	0.35
Phosphorus (mg/dL)	3.5 (0.2)	3.5 (0.2)	0.1 (-0.4,0.6)	0.78	3.4 (0.2)	3.8 (0.2)	0.4 (0.0,0.9)	0.06	-0.4 (-1.1,0.3)	0.23
24,25(OH)$_2D_3$:25(OH)D_3 (pg/ng)	67.8 (9.9)	102.8 (10.0)	35.0 (14.4,55.7)	0.001	119.5 (9.1)	206.1 (9.3)	86.6 (67.5,105.6)	<0.001	-56.2 (-84.4,-28.1)	<0.001
1,25(OH)$_2D_3$:25(OH)D_3 (pg/ng)	1.8 (0.2)	1.1 (0.2)	-0.6 (-1.2,-0.1)	0.02	3.4 (0.2)	1.9 (0.2)	-1.5 (-2.0,-1.0)	<0.001	0.8 (0.1,1.6)	0.03

Vitamin D binding protein (μg/mL)	262.2 (7.5)	250.9 (7.6)	-11.3 (-23.0,0.4)	0.06	249.2 (6.9)	254.9 (7.0)	5.7 (-5.1,16.4)	0.30	-19.7 (-35.8,-3.6)	0.02
Estimated bioavailable 25(OH)D	2.4 (0.4)	5.1 (0.4)	2.7 (1.9,3.4)	<0.001	2.3 (0.3)	4.6 (0.3)	2.3 (1.6,2.9)	<0.001	0.4 (-0.6,1.4)	0.43

Cell contents are mean (SD), mean change (95% confidence interval), difference in mean changes (95% confidence interval), or p-value for interaction comparing change among participants with CKD to change among participants without CKD. Abbreviations: 25(OH)D, 25-hydroxyvitamin D; $25(OH)D_3$, 25-hydroxyvitamin D_3; $25(OH)D_2$, 25-hydroxyvitamin D_2; 25(OH)D total , 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3; $1,25(OH)_2D_2$, 1,25-dihydroxyvitamin D_2; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3, $1,25(OH)_2D_3$; total, 1,25-dihydroxyvitamin D_2 and 1,25-dihydroxyvitamin D_3; $24,25(OH)_2D_3$, 24,25-dihydroxyvitamin D_3; PTH, parathyroid hormone; FGF-23, fibroblast growth factor 23; $24,25(OH)_2D_3$:25(OH)D, the ratio of 24,25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3; $1,25(OH)_2D_3$:$25(OH)D_3$, the ratio of 1,25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3.

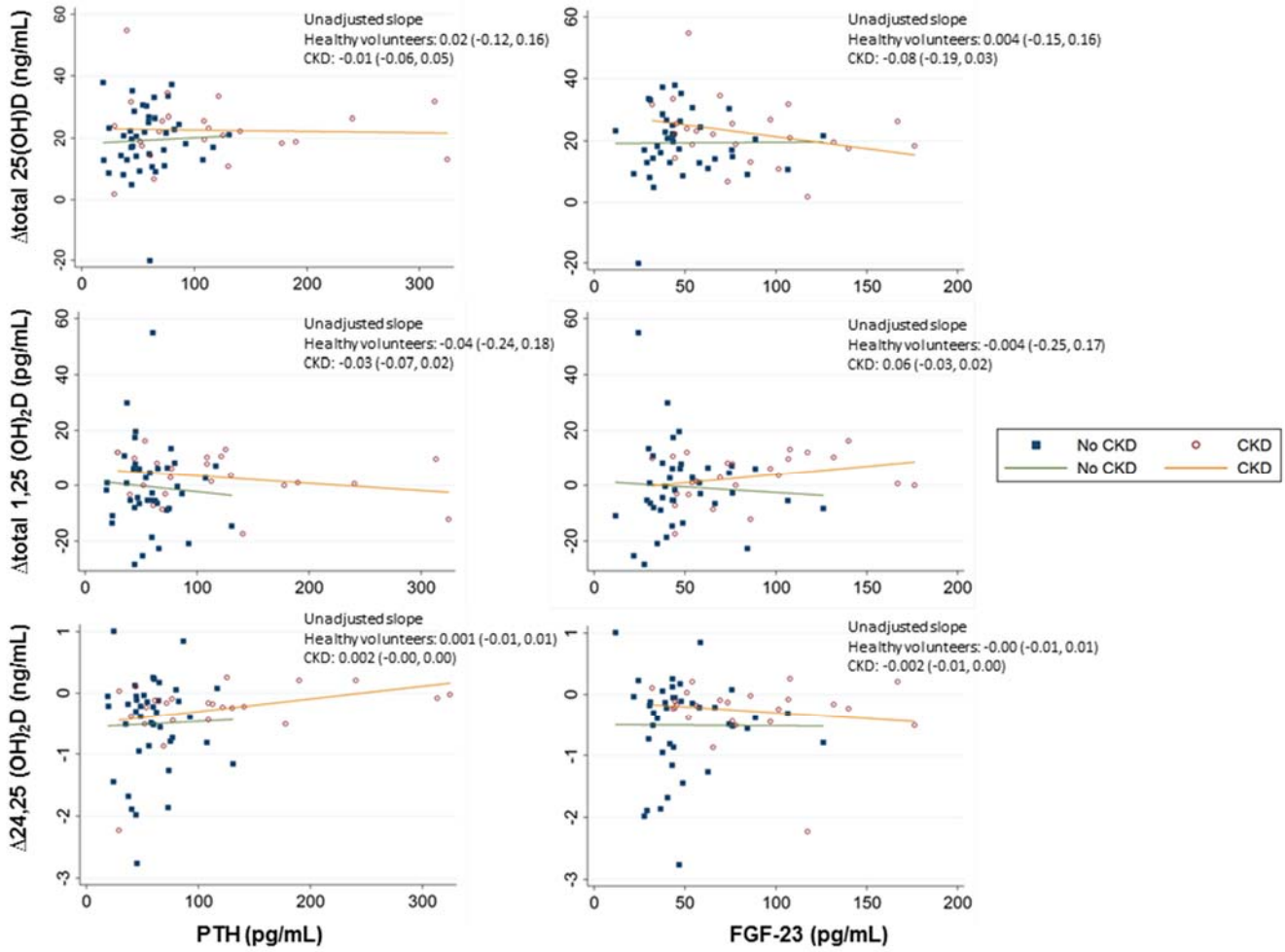
Supplementary Table S3. Percentage Change in Mineral Metabolism Biomarkers by CKD status, before and after treatment.

Biomarker	Mean change with treatment (%, with SD)		Difference in mean change with treatment by CKD status (%, with 95% CI)	p-value interaction
	CKD (eGFR <60 mL/min/1.73m^2)	Non-CKD (eGFR ≥60 mL/ min/1.73m^2)		
25(OH)D total (ng/mL)	135.5 (73.7)	131.8 (144.9)	3.7 (-55.8,63.3)	0.90
25(OH)D_3 (ng/mL)	-41.9 (12.8)	-49.1 (18.6)	7.3 (-0.9,15.4)	0.08
25(OH)D_2 (ng/mL)	7426.6 (7672.2)	10653.0 (12925.9)	-3226.4 (-8684.7,2232.0)	0.25
1,25(OH)$_2$D total (pg/mL)	18.4 (31.0)	7.8 (68.7)	10.6 (-17.1,38.4)	0.45
1,25(OH)$_2D_3$ (pg/mL)	-62.1 (16.8)	-61.5 (43.1)	-0.6 (-17.8,16.5)	0.94
1,25(OH)$_2D_2$ (pg/mL)	3526.2 (3405.7)	3997.0 (5225.6)	-470.9 (-2729.0,1787.3)	0.68
24,25 (OH)$_2D_3$ (ng/mL)	-10.6 (31.7)	-12.0 (37.2)	1.4 (-15.9,18.7)	0.87
PTH (pg/mL)	2.3 (39.5)	-13.8 (30.1)	16.1 (-0.5,32.8)	0.06
FGF-23 (pg/mL)	3.9 (29.0)	10.4 (35.5)	-6.6 (-22.6,9.4)	0.42
Calcium (mg/dL)	1.9 (17.8)	1.7 (10.1)	0.2 (-7.1,7.5)	0.96
Phosphorus (mg/dL)	5.6 (23.6)	10.8 (39.1)	-5.2 (-22.0,11.6)	0.54
24,25(OH)$_2D_3$:25(OH)D (pg/ng)	53.8 (41.0)	87.0 (118.3)	-33.2 (-79.8,13.3)	0.16
1,25(OH)$_2D_3$:25(OH)D_3(pg/ng)	-34.0 (29.8)	-22.3 (103.6)	-11.7 (-52.0, 28.6)	0.57
Vitamin D binding protein (µg/mL)	-2.5 (12.1)	2.4 (11.5)	-4.9 (-10.7, 0.9)	0.10
Estimated bioavailable 25(OH)D ng/mL	160.1 (120.4)	138.2 (154.0)	21.9 (-49.5, 93.3)	0.55

Cell contents are mean percentage change (SD) with treatment and difference in mean percentage change (95% confidence interval), or p-value for interaction comparing change among participants with CKD to change among participants without CKD. Abbreviations: 25(OH)D, 25-hydroxyvitamin D; 25(OH)D_3, 25-hydroxyvitamin D_3; 25(OH)D_2, 25-hydroxyvitamin D_2; 25(OH)D total , 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3; 1,25(OH)$_2D_2$, 1,25-dihydroxyvitamin D_2; 1,25(OH)$_2D_3$, 1,25-dihydroxyvitamin D_3, 1,25(OH)$_2D_3$; total, 1,25-dihydroxyvitamin $D_2$and

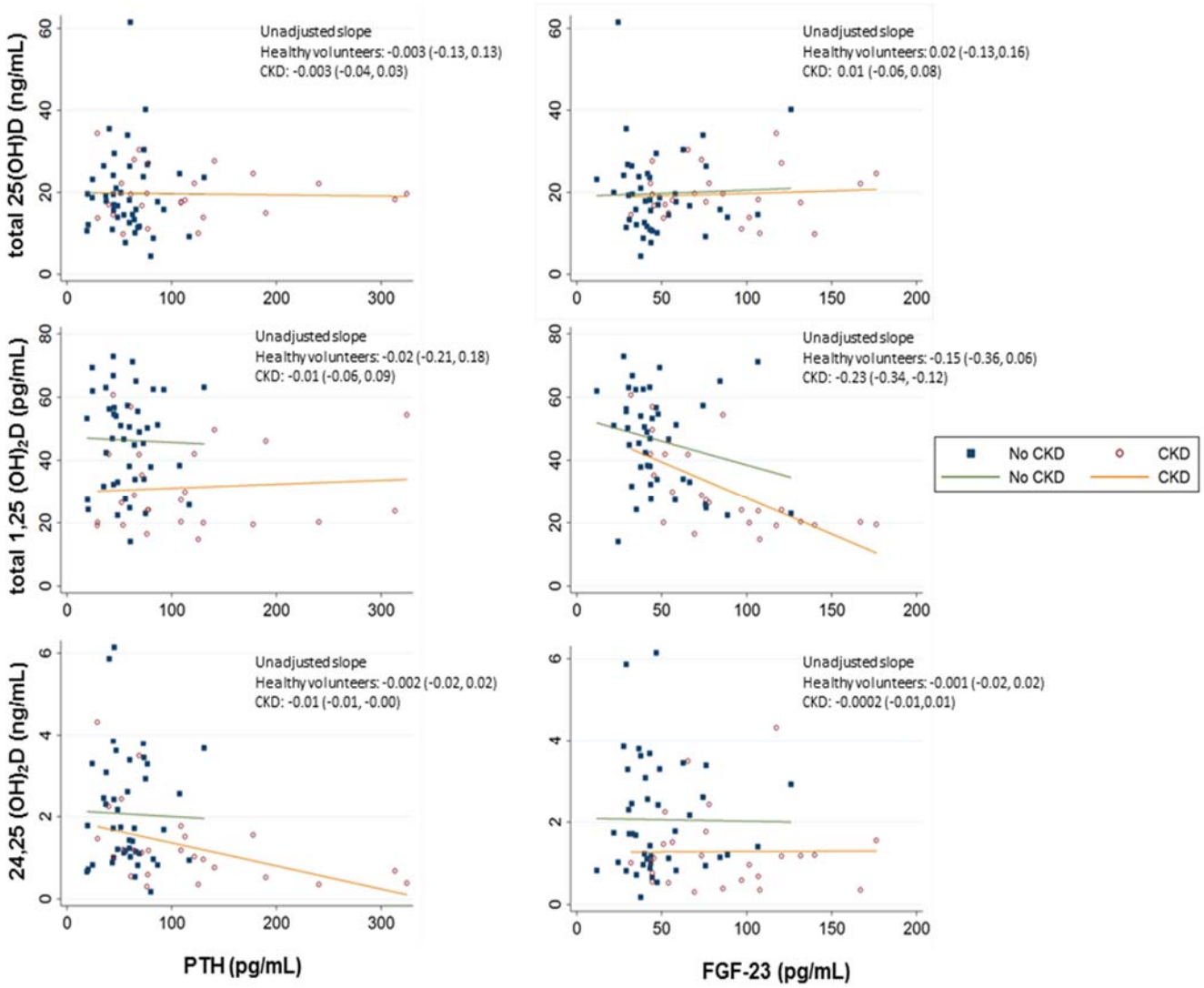
1,25-dihydroxyvitamin D_3; 24,25$(OH)_2D_3$, 24,25-dihydroxyvitamin D_3; PTH, parathyroid hormone; FGF-23, fibroblast growth factor 23; 24,25$(OH)_2D_3$:25(OH)D, the ratio of 24,25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3; 1,25$(OH)_2D_3$:25$(OH)D_3$, the ratio of 1,25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3.

Supplementary Figure 1: Relationships of baseline plasma PTH and FGF-23 concentrations with change in mineral metabolism biomarkers with treatment, by CKD status.



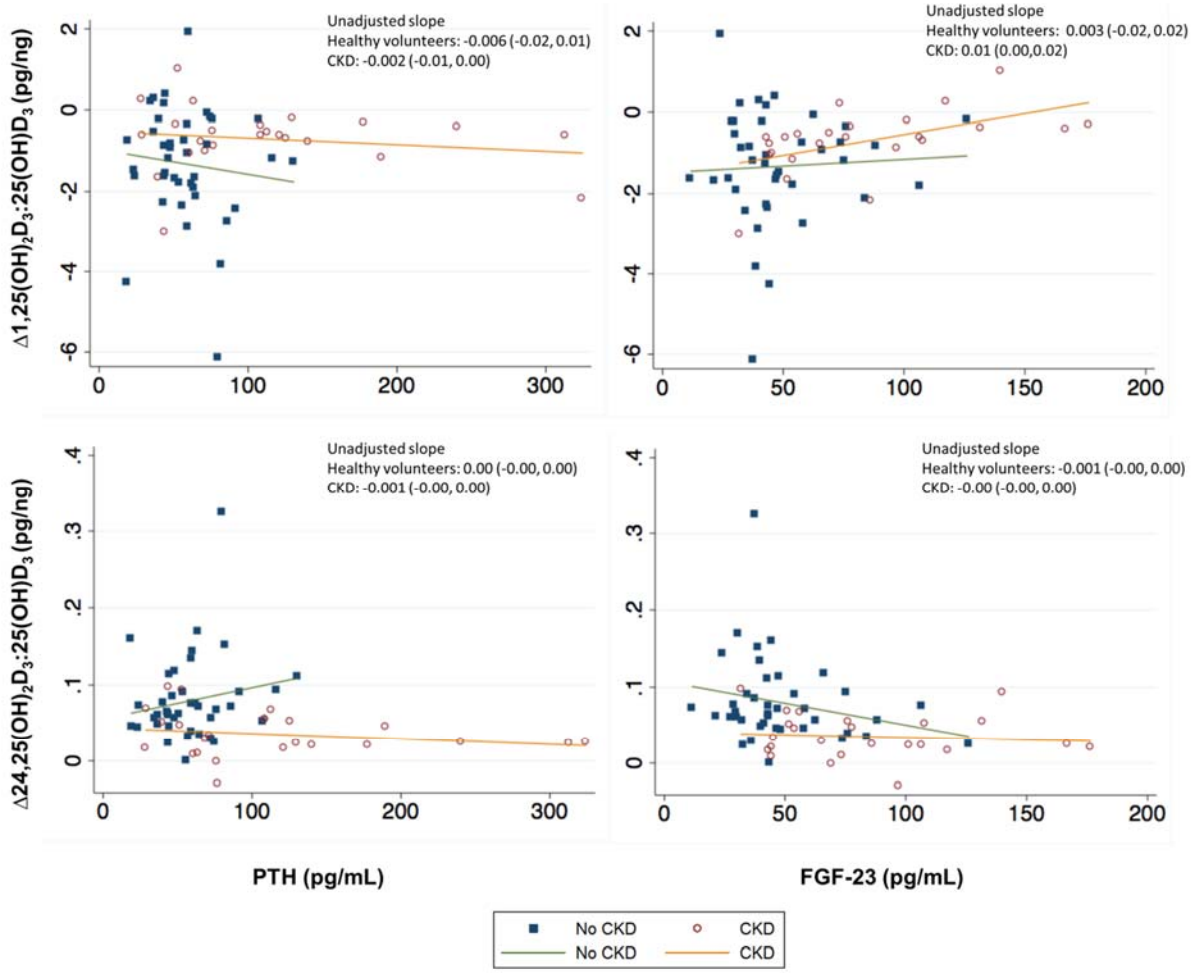
Relationships of change in total 25-hydroxyvitamin D; change in total 1,25 dihydroxyvitamin D and change in total 24,25-dihydroxyvitamin D_3 with baseline PTH and baseline FGF-23. Blue boxes represent individual values for participants without CKD and red circles individual values for participants with CKD. Solid lines represent mean concentrations using linear fit lines. Unadjusted slopes with 95% confidence intervals represented on each graph. Abbreviations: 25(OH)D, 25-hydroxyvitamin D; 1,25-dihydroxyvitamin D; $1,25(OH)_2D$; $24,25(OH)_2D_3$, 24,25-dihydroxyvitamin D_3; PTH, parathyroid hormone; FGF-23, fibroblast growth factor 23.

Supplementary Figure 2: Relationships of baseline plasma PTH and FGF-23 concentrations with baseline mineral metabolism biomarkers, by CKD status.



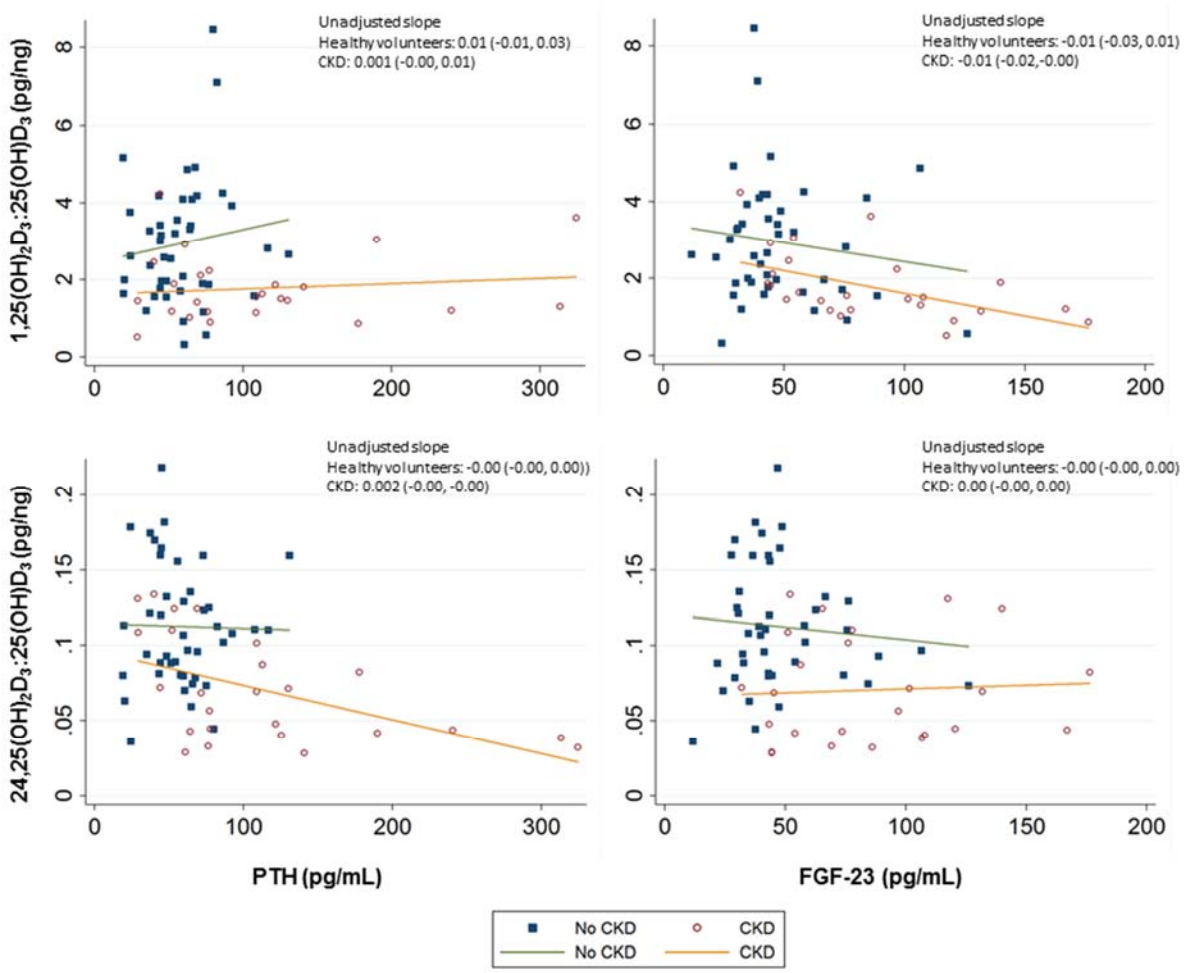
Relationships of baseline total 25-hydroxyvitamin D; total 1,25 dihydroxyvitamin D and total 24,25-dihydroxyvitamin D_3 with baseline PTH and baseline FGF-23. Blue boxes represent individual values for participants without CKD and red circles individual values for participants with CKD. Solid lines represent mean concentrations using linear fit lines. Unadjusted slopes with 95% confidence intervals represented on each graph. Abbreviations: 25(OH)D, 25-hydroxyvitamin D; 1,25-dihydroxyvitamin D; $1,25(OH)_2D$; $24,25(OH)_2D_3$, 24,25-dihydroxyvitamin D_3; PTH, parathyroid hormone; FGF-23, fibroblast growth factor 23.

Supplementary Figure 3: Relationships of baseline plasma PTH and FGF-23 concentrations with change in vitamin D metabolic ratios with treatment, by CKD status.



Relationships of changes in concentrations of $1,25(OH)_2D_3$:$25(OH)D_3$ and $24,25(OH)_2D_3$:25(OH)D vitamin D metabolic ratios with baseline PTH and baseline FGF-23. Blue boxes represent individual values for participants without CKD and red circles individual values for participants with CKD. Solid lines represent mean concentrations using linear fit lines. Unadjusted slopes with 95% confidence intervals represented on each graph. Abbreviations: PTH, parathyroid hormone; FGF-23, fibroblast growth factor 23; $24,25(OH)_2D_3$:$25(OH)D$, the ratio of 24,25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3; $1,25(OH)_2D_3$:$25(OH)D_3$, the ratio of 1,25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3

Supplementary Figure 4: Relationships of baseline plasma PTH and FGF-23 concentrations with change in baseline vitamin D metabolic ratios, by CKD status.



Relationships of baseline $1,25(OH)_2D_3$:25(OH)D_3 and $24,25(OH)_2D_3$:25(OH)D vitamin D metabolic ratios with baseline PTH and baseline FGF-23. Blue boxes represent individual values for participants without CKD and red circles individual values for participants with CKD. Solid lines represent mean concentrations using linear fit lines. Unadjusted slopes with 95% confidence intervals represented on each graph. Abbreviations: PTH, parathyroid hormone; FGF-23, fibroblast growth factor 23; $24,25(OH)_2D_3$:25(OH)D, the ratio of 24,25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3; $1,25(OH)_2D_3$:25(OH)D_3, the ratio of 1,25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3

Supplementary Table S4: Vitamin D Binding Protein Haplotypes of Study Participants, by CKD status.

Haplotype	CKD eGFR<60 mL/min/1.73m^2 N=43	Non-CKD eGFR≥60 mL/min/1.73m^2 N=25
Gc2/Gc1f	2 (8)	4 (9)
Gc1f/Gc1s	3 (12)	8 (19)
Gc1s/Gc1s	6 (24)	11 (26)
Gc2/Gc1s	8 (32)	9 (21)
Gc1f/Gc1f	5 (20)	6 (14)
Gc2/Gc2	1 (4)	5 (12)

Cell contents are presented as N (%), frequency of haplotype by CKD status. Data is missing for 1 participant in the CKD group.