SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Deuterated 25-hydroxyvitamin D_3

Deuterated 25-hydroxyvitamin D_3 was specially designed for this study and manufactured at Sigma-Aldrich ISOTEC, Stable Isotope Division (Miamisburg, OH). It was formulated as single use vials in 10% ethanol and 60% propylene glycol at SRI International (Menlo Park, CA), where stability testing verified concentration within 10% of target and suitability for human injection according to GCP and FDA standards.

Covariates

Diabetes was defined by self-report, fasting blood glucose > 126 mg/dL, non-fasting blood glucose > 200 mg/dL, hemoglobin A1c $\geq$ 6.5% or use of glucose-lowering medications. Blood pressure was measured three times 5 minutes apart on an automated sphygmomanometer, and were averaged for analysis. Hypertension was defined by self-report, systolic blood pressure $\geq$ 140 mmHg, diastolic blood pressure $\geq$ 90 mmHg, or use of antihypertensive medications. Current medication and supplement use were ascertained from pill bottles and computerized medication lists. Height and weight were measured during the screening visit. Estimated blood volume (EBV) was calculated using the Nadler equation: for men, EBV = (0.3669 x (height in meters)^3) + (0.03219 x weight in kilograms) + 0.641; for women, EBV = (0.3561 x (height in meters)^3) + (0.03308 x weight in kilograms) + 0.1833.

SUPPLEMENTAL TABLES

Supplemental Table 1. Analytical variability of the assays, expressed as between-batch coefficient of variation (CV%) of vitamin D metabolites

Metabolite	CV (%)	Concentration Range Tested
$25(OH)D_2$	3.45% - 15.23%	1.33 – 40.82 ng/mL
$25(OH)D_3$	1.72% - 3.30%	14.94 – 41.25 ng/mL
$1,25(OH)_2D_2$	5.04% - 7.65%	1.00 – 52.60 pg/mL
$1,25(OH)_2D_3$	3.00% - 5.40%	27.31 – 82.69 pg/mL
$24,25(OH)_2D_3$	1.96% - 6.23%	1.15 – 3.44 ng/mL
d-$25(OH)D_3$	2.71% - 24.86%	0.76 – 7.81 ng/mL
d-$24,25(OH)_2D_3$	16.9% - 76.4%	0.02 – 0.20 ng/mL

25(OH)D = 25-hydroxyvitamin D; $1,25(OH)_2D$ = 1,25-dihydroxyvitamin D; $24,25(OH)_2D_3$ = 24,25-dihydroxyvitamin D_3; d-$25(OH)D_3$ = deuterated 25-hydroxyvitamin D_3; d-$24,25(OH)_2D_3$ = deuterated 24,25-dihydroxyvitamin D_3

Supplemental Table 2. Characteristics of the vitamin D_3 supplemented cohort

	Healthy controls (n = 10)	CKD (n = 8)
Age (years), mean (SD)	60 ± 6	69 ± 7
Female, n (%)	4 (40%)	2 (25%)
Race, n (%)		
White	4 (40%)	4 (50%)
Black	6 (60%)	4 (50%)
Hypertension, n (%)	3 (30%)	6 (75%)
Diabetes, n (%)	1 (10%)	5 (63%)
Ever smoker, n (%)	5 (50%)	2 (25%)
RAAS-I use, n (%)	1 (10%)	5 (63%)
Statin use, n (%)	1 (10%)	5 (63%)
Systolic BP (mmHg), mean (SD)	118 (9)	118 (26)
BMI (kg/m^2), mean (SD)	27.4 (3.7)	29.7 (5.0)
EBV (L), mean (SD)	5.0 (0.7)	5.6 (0.9)
Total 25(OH)D (ng/mL), mean (SD)		
Baseline	17 (6)	26 (5)
After vitamin D_3 supplementation	33 (6)	40 (10)
PTH (pg/mL), median (IQR)		
Baseline	62 (45, 66)	102 (77, 165)
After vitamin D_3 supplementation	41 (34, 54)	79 (62, 151)
FGF-23 (pg/mL), median (IQR)		
Baseline	55 (51, 67)	134 (105, 164)
After vitamin D_3 supplementation	54 (50, 65)	140 (85, 183)
Calcium (mg/dL), mean (SD)		
Baseline	9.2 (0.3)	9.2 (0.3)
After vitamin D_3 supplementation	9.3 (0.2)	9.2 (0.3)
VDBG (μg/mL), mean (SD)	191 (26)	205 (34)
eGFR ($ml/min/1.73m^2$), mean (SD)	89 (12)	37 (13)
Urine albumin/creatinine (mg/g), median (IQR)	0 (0, 28)	96 (22, 253)

eGFR = estimated glomerular filtration rate; CKD = chronic kidney disease; RAAS-I = renin-angiotensin-aldosterone inhibitor; BP = blood pressure; BMI = body mass index; EBV =

estimated blood volume; 25(OH)D = 25-hydroxyvitamin D; PTH = parathyroid hormone; FGF-23 = fibroblast growth factor-23; VDBG = vitamin D binding globulin; SD = standard deviation; IQR = interquartile range

Supplemental Table 3. Sensitivity analysis of associations of eGFR and race with 25(OH)D clearance in the full study population versus excluding two outlier participants

	β coefficient (95% CI) of 25(OH)D clearance (mL/day) per 10 mL/min/1.73m^2 eGFR lower	
	Outliers excluded (n = 85)	Full study population (n = 87)
Unadjusted model		
All participants	-14 (-19, -8)	-13 (-20, -6)
Among black participants	-20 (-29, -12)	-20 (-29, -12)
Among white participants	-10 (-16, -4)	-10 (-19, 0.2)
Adjusted model*		
All participants	-17 (-21, -12)	-17 (-23, -11)
Among black participants	-21 (-30, -12)	-21 (-30, -12)
Among white participants	-15 (-20, -10)	-16 (-23, -8)

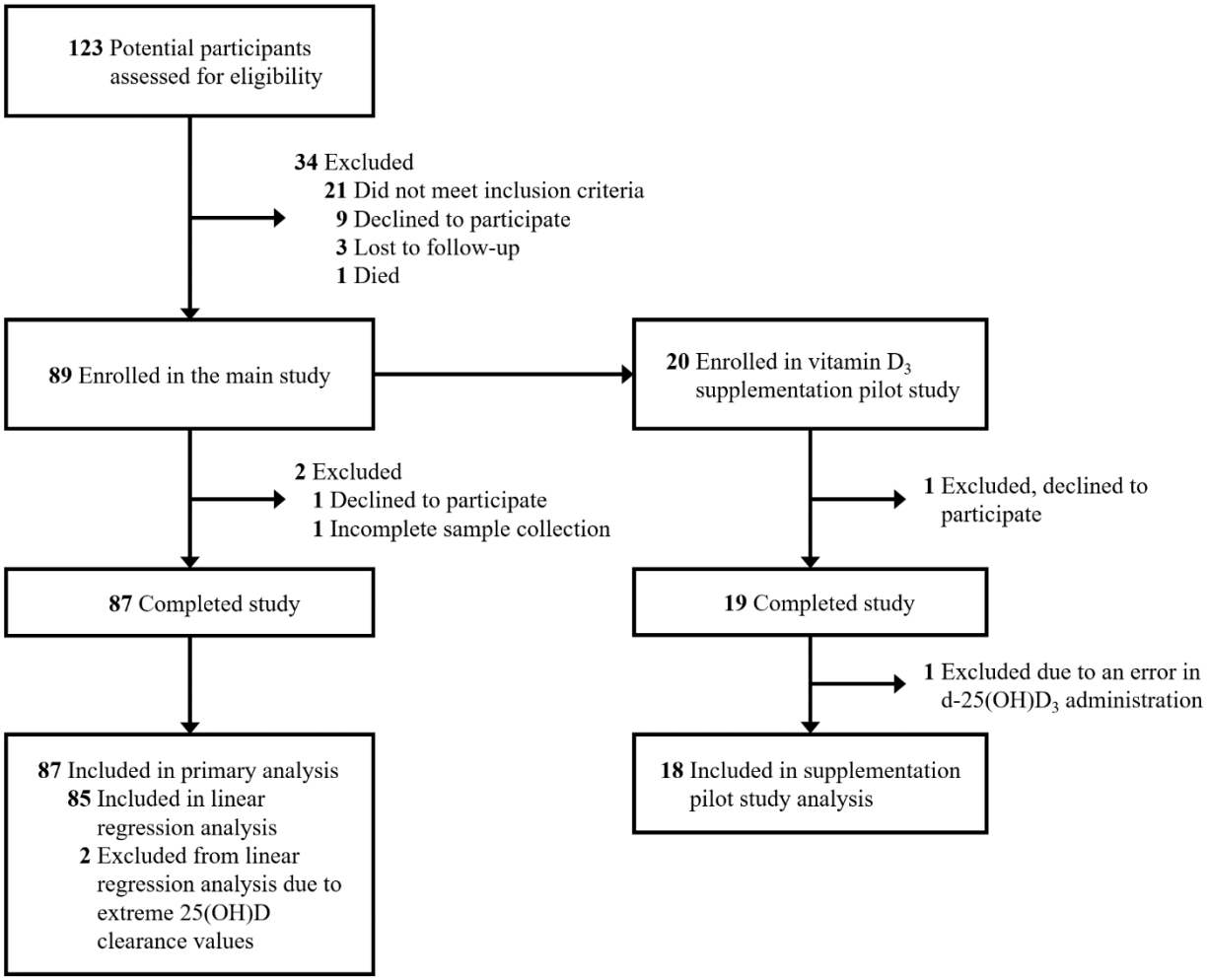
	β coefficient (95% CI) of 25(OH)D clearance (mL/day) comparing black with white race	
	Outliers excluded (n = 85)	Full study population (n = 87)
Unadjusted model		
All participants	41 (-1, 83)	23 (-35, 80)
Among healthy controls	92 (41, 144)	77 (6, 148)
Adjusted model**		
All participants	22 (-12, 56)	-11 (-55, 34)
Among healthy controls	71 (16, 125)	26 (-32, 84)

eGFR = estimated glomerular filtration rate; 25(OH)D = 25-hydroxyvitamin D

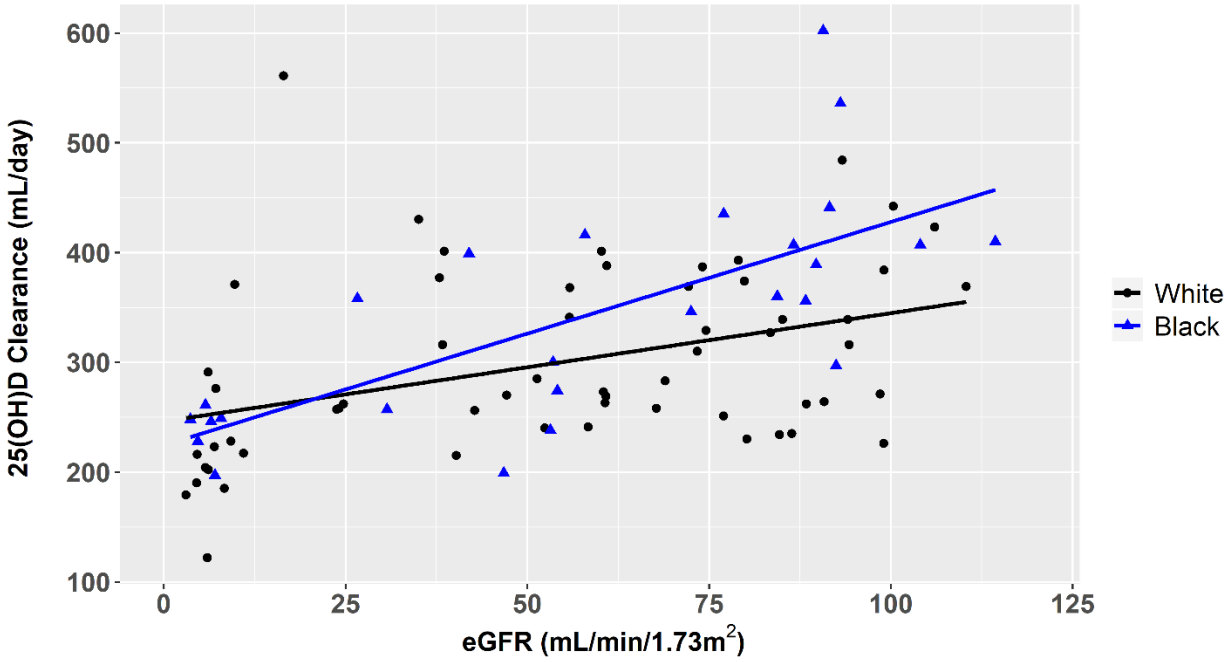
*Adjusted for age, sex, race and estimated blood volume

**Additionally adjusted for eGFR

SUPPLEMENTAL FIGURES



Supplemental Figure 1. Participant flow in the Clearance of 25-hydroxyvitamin D in Chronic Kidney Disease Study. 25(OH)D = 25-hydroxyvitamin D; d-25(OH)D_3 = deuterated 25-hydroxyvitamin D_3



Supplemental Figure 2. Relationship between eGFR with 25-hydroxyvitamin D clearance by race. eGFR = estimated glomerular filtration rate; 25(OH)D = 25-hydroxyvitamin D

Supplemental Figure 3. Linear regression depicting the relationship of 25-hydroxyvitamin D response to vitamin D_3 supplementation with mean 25-hydroxyvitamin D clearance in the vitamin D_3 supplemented cohort. 25(OH)D = 25-hydroxyvitamin D; CKD = chronic kidney disease

*Total 25(OH)D after vitamin D_3 supplementation – total 25(OH)D before vitamin D_3 supplementation

**From the parent study and vitamin D_3 supplementation sub-study