Supplementary Online Content

Hansen KE, Johnson RE, Chambers KR, et al. Treatment of vitamin D insufficiency in postmenopausal women: a randomized clinical trial. *JAMA Intern Med.* Published online August 3, 2015. doi:10.1001/jamainternmed.2015.3874.

This supplementary material has been provided by the authors to give readers additional information about their work.

eFigure 1: Randomization Strategy and Allocation Concealment

Baseline Calcium Absorption Study → Randomization, stratified by high or normal parathyroid hormone and calcium intake ≤ or >1,000 mg/day →

Treatment Arm	Loading Dose		Maintenance Dose
Placebo	Daily white placebo pill x 15 days Daily yellow placebo pill x 15 days	→	White placebo pill daily Yellow placebo pill every 15 days
Low-Dose Vitamin D_3	Daily white vitamin D pill x 15 days Daily yellow placebo pill x 15 days	→	White vitamin D pill daily Yellow placebo pill every 15 days
High-Dose Vitamin D_3	Daily white placebo pill x 15 days Daily yellow vitamin D pill x 15 days	→	White placebo pill daily Yellow vitamin D pill every 15 days

Low-dose vitamin D was administered in white capsules that each contained 800 international units (IU). High-dose vitamin D was administered in yellow capsules that each contained 50,000 IU. University of Wisconsin Professor Hector DeLuca independently verified the content of white and yellow capsules, prior to use in the trial. The University of Wisconsin Pharmaceutical Research Center randomized subjects into treatment arms, monitored serum vitamin D levels during the trial, and adjusted high-dose capsule doses to maintain serum 25(OH)D levels ≥30 ng/mL, with sham adjustments of yellow placebo pills in ~5% of the other two treatment arms to preserve blinding. All subjects and all study team members were blinded to treatment assignment.

eTable 1. Adherence to Study Pills by Treatment Allocation

	Placebo n= 73 of 76	Low-Dose Vitamin D n= 74 of 75	High-Dose Vitamin D n= 74 of 79	*P* value across treatment arms
White Pills	99.5 (97.7, 100.0)	99.7 (98.8, 100.0)	99.5 (98.4, 100.0)	0.38
Yellow Pills	100.0 (100.0, 100.0)	100.0 (100.0, 100.0)	100.0 (100.0, 100.0)	0.63

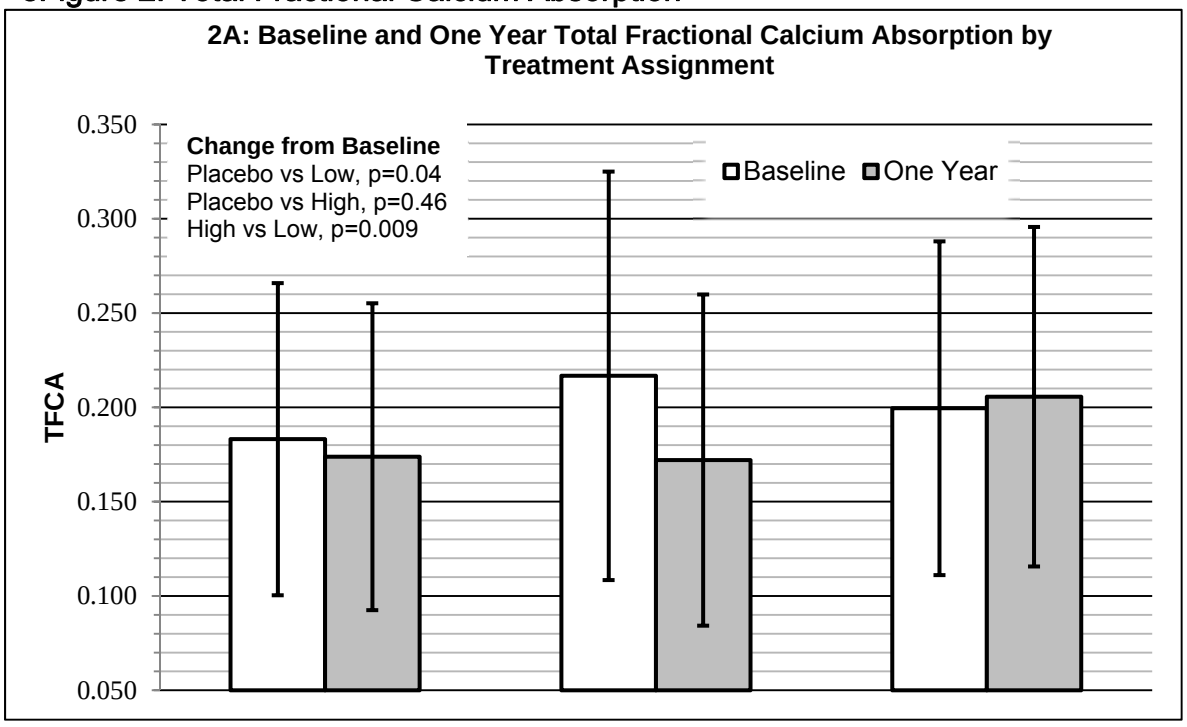
Adherence data was skewed based on analysis using the Shapiro-Wilk test, and is therefore summarized using the median (25th, 75th interquartile range) and analyzed using the Kruskal-Wallace test. White pills contained either placebo or 800 IU vitamin D_3. Yellow pills contained either placebo or 50,000 IU vitamin D_3.

eTable 2. Subjects' Dietary Habits

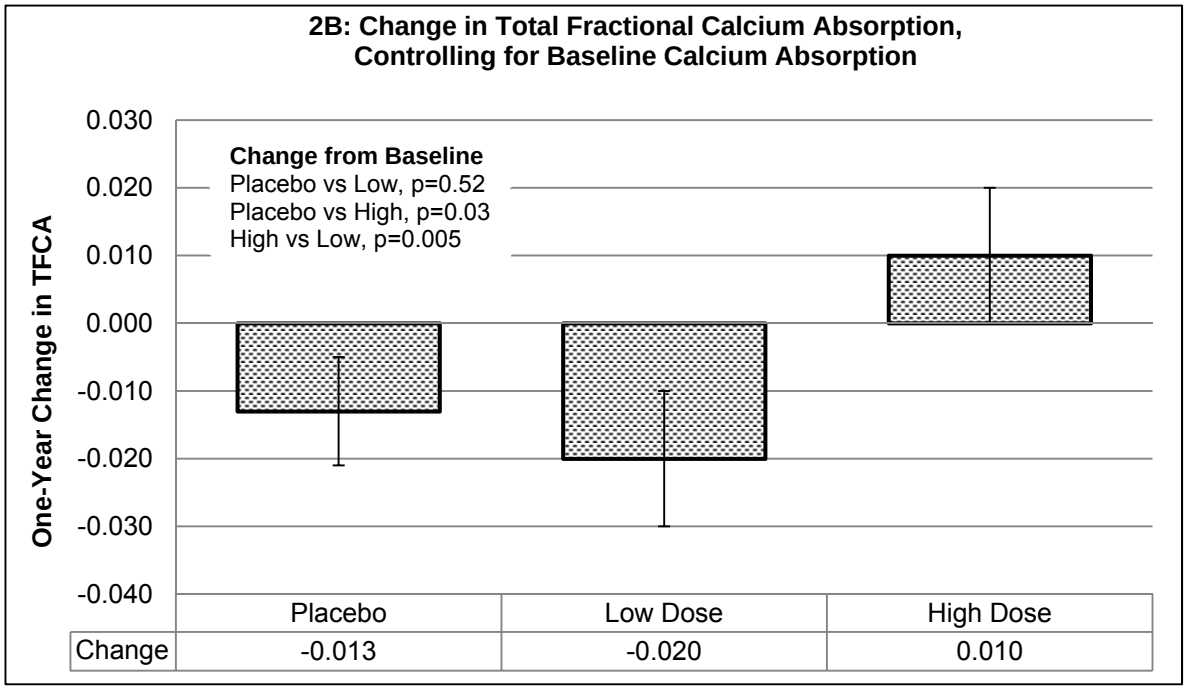
Dietary Habits	Baseline	One Year	Adjusted *P* value[a]
Kilocalories, kcal/day	1842 (1544, 2199)	1796 (1493, 2112)	0.67
Carbohydrates, g/day	222 (175, 264)	215 (173, 261)	0.69
Protein, g/day	74 (61, 86)	73 (61, 86)	0.85
Fat, g/day	72 (60, 91)	71 (55, 85)	0.24
Fiber, g/day	19 (15, 25)	19 (14, 25)	0.69
Calcium Intake, mg/day[b]	970 (752, 1223)	918 (724, 1197)	0.67
Iron, mg/day	13 (10, 16)	13 (10, 17)	0.86
Magnesium, mg/day	305 (248, 372)	294 (238, 360)	0.53
Phosphorus, mg/day	1308 (1081, 1475)	1285 (1057, 1510)	0.72
Potassium, mg/day	2786 (2326, 3263)	2750 (2281, 3329)	0.86
Vitamin D, IU/day	199 (115, 267)	176 (119, 265)	0.86
Oxalate, servings/day	0.9 (0.4, 1.8)	0.9 (0.4, 1.7)	0.86

Here, we summarize dietary habits based on food records for 221 subjects who completed the study. Subjects' dietary habits were summarized using the median (25^{th}, 75^{th} interquartile range) and analyzed using the Wilcoxon signed-rank test for paired (within-subject) data. [a]P-values are adjusted to control the false discovery rate, using the Benjamini and Hochberg method. [b]Calcium intake was reported as the sum of dietary and supplemental intake.

eFigure 2: Total Fractional Calcium Absorption



	Placebo	Low Dose	High Dose
Baseline	0.183 (0.083)	0.217 (0.108)	0.200 (0.088)
One Year	0.174 (0.081)	0.172 (0.088)	0.206 (0.090)



eFigure 2: Total Fractional Calcium Absorption by Treatment Assignment
Total fractional calcium absorption (TFCA) was measured one day prior to randomization and again ~365 days later, using the dose-corrected ratios of two stable isotopes in a 24-hour urine collection. 3A: TFCA, presented as the median and interquartile range, differed significantly between treatment arms (adjusted p=0.02). TFCA increased significantly in the high-dose compared to the low-dose vitamin D arm (p=0.009), with no significant difference between high-dose and placebo arms (p=0.46). By chance, the placebo arm had higher baseline TFCA. 3B: When the change in TFCA was normalized for baseline TFCA, data could be summarized using the mean and standard error and was significantly different between treatment arms (adjusted p=0.01). The change in TFCA was not significantly different between placebo and low-dose vitamin D (p=0.52) but was significantly different between placebo and high-dose vitamin D (p=0.03) and between low and high-dose vitamin D (p=0.005).

eTable 3. Multivariate Models of Change in Calcium Absorption

Baseline Variable	Estimate	Standard Error	*P* value
Total Fractional Calcium Absorption	-4.832e-01	6.087e-02	<0.001
Glomerular filtration rate, mL/minute	4.630e-04	2.494e-04	0.07
Estradiol, pg/mL	8.520e-04	3.146e-04	0.01
Serum 25(OH)D, ng/mL	-1.818e-03	8.687e-04	0.04
Serum 25(OH)D at 60 days, ng/mL	7.628e-04	2.112e-04	<0.001
Body Mass Index, kg/m^2	1.950e-03	6.917e-04	0.01
Dietary Sodium, mg/day	-1.115e-05	5.403e-06	0.04
Dietary Fiber, g/day	-1.350e-03	7.970e-04	0.09
Dietary Potassium, mg/day	1.375e-05	8.499e-06	0.11
Dietary Oxalate, servings/day	-6.270e-03	3.869e-03	0.11
Alcohol, g/day	-5.400e-04	3.673e-04	0.14

Together, all variables explained 33.8% of the variance (R^2) for the change in total fractional calcium absorption ($p<0.001$). Removal of dietary potassium, oxalate and alcohol reduced R^2 to 31.8% ($p<0.001$).

eTable 4.Absolute Change in Bone Mineral Density, Content and Trabecular Bone Score between Treatment Arms

Skeletal Site		Placebo n=73 of 76	Low-Dose Vitamin D n=74 of 75	High-Dose Vitamin D n=74 of 79	Overall *P* value	Adjusted *P* value
L1-L4	BMD	0.002 (-0.019, 0.027)	0.001 (-0.026, -0.001)	-0.003 (-0.021, 0.018)	0.58	0.93
	T-score	0 (-0.200, 0.200)	0 (-0.2, 0.2)	0 (-0.2, 0.1)	0.74	0.93
	BMC	0.120 (-1.320, 1.240)	0.305 (-1.292, 1.695)	0.435 (-1.415, 1.388)	0.88	0.93
	TBS	-0.024 (-0.053, 0.014)	-0.017 (-0.040, 0.021)	-0.026 (-0.054, 0.010)	0.38	0.84
Mean total hip	BMD	-0.009 (-0.018, 0.003)	-0.005 (-0.012, 0.002)	-0.002 (-0.012, 0.008)	0.09	0.33
	T-score	-0.100 (-0.100, 0.000)	-0.100 (-0.2, 0)	0 (-0.1, 0.1)	0.06	0.31
	BMC	-0.190 (-0.540, 0.140)	-0.070 (-0.448, 0.215)	0 (-0.290, 0.300)	0.17	0.47
Mean Femoral Neck	BMD	-0.008 (-0.016, -0.001)	-0.009 (-0.020, 0.001)	-0.003 (-0.012, 0.005)	0.03	0.31
Total Body	BMD	-0.006 (-0.017, 0.014)	-0.006 (-0.020, 0.008)	-0.004 (-0.012, 0.003)	0.93	0.93
	T-score	-0.100 (-0.200, 0.100)	-0.100 (-0.2, 0.1)	0 (-0.1, 0)	0.93	0.93
	BMC	-14 (-39, 7)	-16 (-40, 6)	-11 (-32, 7)	0.60	0.93

Data was analyzed using the intent-to-treat principle, in which treatment allocation was assumed to continue throughout the study, despite actual change in serum 25(OH)D levels. Data exhibited a non-normal distribution and was summarized using the mean (interquartile range) and analyzed for between-group changes using the Kruskal-Wallis test. The adjusted p-value denotes Benjamini and Hochberg method to control the false discovery rate. The table presents the summary data from all subjects completing the trial (221 of 230 randomized), with number analyzed shown within each treatment arm. The p-values for within group changes are reported within the same box, just below each observed change.

eTable 5. Changes from Baseline in Bone Turnover Markers by Treatment Assignment [a,b, c, d]

Measure	Visit[c]	Placebo n=46 of 76	Low-Dose Vitamin D n=52 of 75	High-Dose Vitamin D n=51 of 79	Overall *P* value[e]	Adjusted *P* value[f]
C-telopeptide, pg/mL[g]	Baseline	98 (78,110)	97 (80,107)	81 (74,101)	-	-
	30 days	0 (-6, 5)	1 (-3, 4)	2 (-5, 5)	0.61	0.74
	60 days	-1 (-5, 4)	-1 (-5, 2)	0 (-3, 6)	0.21	0.55
	120 days	-2 (-5, 3)	-1 (-5, 2)	1 (-3, 8)	0.10	0.41
	365 days	0 (-5, 5)	0 (-4, 5)	2 (-3, 8)	0.46	0.73
Bone Specific Alkaline Phosphatase[h]	Baseline	89 (33,179)	70 (25,133)	73 (26,137)	-	-
	30 days	5 (-27, 46)	-3 (-38, 43)	12 (-17, 40)	0.33	0.66
	60 days	5 (-58, 37)	6 (-36, 45)	5 (-14, 39)	0.69	0.74
	120 days	-13 (-61, 33)	-1 (-69, 43)	18 (-23, 62)	0.05	0.38
	365 days	2 (-49, 33)	-1 (-53, 59)	12 (-33, 46)	0.74	0.74

[a]We analyzed bone turnover markers in duplicate, for the subset of subjects who arrived at all study visits fasting since midnight and had phlebotomy prior to 10 am.
[b]Data demonstrated outliers and was summarized using the median (25^{th}, 75^{th} interquartile range) and analyzed using the Wilcoxon Rank-Sum test for independent samples.
[c]"Baseline" denotes the measurement at randomization.
[d]We found no significant within-arm changes in bone turnover markers (p-values not shown).
[e]Between-arm differences in bone turnover were analyzed using the Kruskal-Wallis test.
[f]We corrected p-values to control the false discovery rate, using the Benjamini and Hochberg method.
[g]C-telopeptide of type 1 collagen (CTX) was measured by ELISA (NeoBiolab, Cambridge, MA, USA) with a normal range of 250-5,000 pg/mL for pre-menopausal women and an intra and inter-assay CV of 5.6% and 10.5%, respectively.
[h]Bone specific alkaline phosphatase (BSAP) was measured using the Novateinbio ELISA kit (Cambridge, MA, USA) with a normal range of 25-800 U/L and an intra and inter-assay CV of 5.4% and 13%, respectively.

eTable 6. Adverse Events of Interest by Treatment Allocation

Adverse Event of Interest [a,b]	Placebo n=76	Low-Dose Vitamin D n=75	High-Dose Vitamin D n=79	Adjusted *P* value[c]
Hypercalcemia[d]	0	2	0	0.44
Hypercalciuria[e]	1	1	7	0.19
Nephrolithiasis[f]	0	1	0	0.88
Fall	33	37	35	1.00
Fracture	4	2	2	1.00
Hospitalization	1	1	1	1.00
Death	0	0	0	1.00

[a]At each visit, we asked subjects about incident nephrolithiasis, fracture, fall, infection or hospitalization. Rates of each event were calculated by counting the number of post-randomization visits per treatment arm (placebo=373, low-dose=370 and high-dose= 375 visits post-randomization).

[b]All other adverse events are listed in Table C.

[c]Reflects the p-value corrected to control the false discovery rate, using the Benjamini and Hochberg method.

[d]Serum calcium was measured at 0, 30, 60, 120, 240 and 365 days post-randomization and hypercalcemia defined as a serum calcium ≥10.4 mg/dL.

[e]24-hour urine calcium levels were measured at 0, 60, 120, and 365 days post-randomization. Hypercalciuria was defined as >400 mg calcium in a 24-hour urine collection.

[f]A subject underwent abdominal computed tomography imaging to assess postmenopausal bleeding, and nephrolithiasis was found incidentally. As the subject had no prior abdominal imaging, the timing of nephrolithiasis could not be determined.

eTable 7. Safety Laboratory Studies by Treatment Assignment

Laboratory Test	Visit Day	Placebo n=76	n per test	Low-Dose vitamin D n=75	n per test	High-Dose Vitamin D n=79	n per test	*Adjusted P* value
Serum Calcium, mg/dL	0	8.9 ± 0.3	76	8.9 ± 0.3	75	8.9 ± 0.3	79	1.00
	30	9.1 ± 0.4	76	9.1 ± 0.4	75	9.2 ± 0.3	79	0.46
	60	9.2 ± 0.3	76	9.1 ± 0.4	74	9.2 ± 0.3	76	0.84
	120	9.1 ± 0.3	75	9.1 ± 0.3	73	9.1 ± 0.3	73	0.84
	240	9.1 ± 0.4	73	9.1 ± 0.3	73	9.2 ± 0.3	73	0.29
	365	8.9 ± 0.3	73	8.9 ± 0.3	74	9.0 ± 0.3	74	0.12
24-hour Urine Calcium, mg	0	167 ± 78	76	171 ± 72	75	198 ± 125	78	0.21
	60	152 ± 85	73	161 ± 71	74	202 ± 87	76	**<0.01**
	120	156 ± 77	74	181 ± 87	72	194 ± 87	72	0.12
	365	166 ± 75	73	176 ± 69	73	198 ± 90	74	0.12
Serum Phosphorus, mg/dL	0	3.5 ± 0.5	76	3.4 ± 0.4	75	3.4 ± 0.4	79	0.60
	365	3.5 ± 0.4	73	3.4 ± 0.4	74	3.4 ± 0.4	74	0.89

Laboratory data are summarized using the mean ± standard deviation and compared across treatment groups using analysis of variance, with correction of p-values to control the false discovery rate using the Benjamini and Hochberg method. The number of subjects randomized into each treatment group is provided in the second row, and the number of subjects undergoing each safety laboratory test is shown to the right of each test. For data demonstrating a significant difference across the three treatment arms, we next analyzed pair-wise differences with 95% confidence intervals and summarize those in Table 6. SI conversion factors: To convert calcium to mmol/L, multiply values by 0.25. To convert urine calcium to mmol, multiply values by 0.025. To convert phosphorus to mmol/L, multiply values by 0.323.

eTable 8. Pairwise Differences in Serum and Urine Calcium Levels by Study Visit

Laboratory Study	Visit Day	Placebo vs High Dose	*P* value	Placebo vs Low Dose	*P* value	High vs Low Dose	*P* value
Serum Calcium, mg/dL	365	-0.14 (-0.27, -0.00)	0.04	-0.02 (-0.15, +0.11)	0.93	-0.12 (-0.25, +0.02)	0.10
24-hour Urine Calcium, mg	60	-50 (-82, -19)	0.001	-9 (-41, +22)	0.77	-41 (-72, -10)	0.007
	120	-38 (-70, -5)	0.02	-25 (-58, +8)	0.17	-13 (-46, +20)	0.63
	365	-32 (-63, -2)	0.04	-10 (-41, +20)	0.70	-22 (-53, +9)	0.21

Between-treatment differences in serum and urine calcium were analyzed as absolute differences (95% confidence interval) and associated p-values, using the Tukey test. SI conversion factors: To convert calcium to mmol/L, multiply values by 0.25. To convert urine calcium to mmol, multiply values by 0.025.

eTable 9. Adverse Events by Treatment Allocation

Disorder	Placebo n=76	Low-Dose Vitamin D n=75	High-Dose Vitamin D n=79	Adjusted *P* value
Blood and lymphatic system	1	3	1	1.00
Cardiac	6	12	9	1.00
Congenital	0	0	0	1.00
Ear and labyrinth	0	1	0	1.00
Endocrine	5	3	4	1.00
Eye	2	5	3	1.00
Gastrointestinal	22	23	18	1.00
General	6	3	3	1.00
Hepatobiliary	0	1	1	1.00
Immune system	8	7	2	1.00
Infection and infestation	80	73	70	1.00
Injury, poisoning, procedural	1	0	3	1.00
Investigations	0	0	0	1.00
Metabolism and nutrition	2	3	4	1.00
Musculoskeletal, connective tissue	102	98	83	1.00
Neoplasm, benign, malignant	4	0	4	1.00
Nervous system	11	5	11	1.00
Pregnancy, peuperium, perinatal	0	0	0	1.00
Psychiatric	6	3	3	1.00
Renal and urinary	3	4	8	1.00
Reproductive and breast	2	1	4	1.00
Respiratory, thoracic, mediastinal	9	1	7	0.86
Skin and subcutaneous tissue	11	8	9	1.00
Social circumstances	0	0	0	1.00
Surgical and medical procedures	13	12	8	1.00
Vascular disorders	0	0	0	1.00

Fisher exact tests were performed across treatment groups, with the denominator representing the number of study visits after randomization, per treatment arm. The number of post-randomization study visits was similar across groups (373 in placebo, 370 in low and 375 in high-dose groups).
P-values reflect correction to control the false discovery rate, using the Benjamini and Hochberg method.