Published in final edited form as:

Menopause. 2016 June ; 23(6): 645–652. doi:10.1097/GME.0000000000000600.

Effects of vitamin D supplementation during weight loss on sex hormones in postmenopausal women

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Abstract

Objective—Compare the effects of vitamin D₃ supplementation versus placebo on serum sex hormones in postmenopausal women completing a 12-month diet + exercise weight loss program.

Methods—218 overweight or obese women (50–75 y) with serum 25-hydroxyvitamin D 10 – <32 ng/mL (‘insufficient’) were randomized to either: i) weight loss + 2000 IU/day oral vitamin

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Trial Registration: www.clinicaltrials.gov Identifier NCT01240213

Financial disclosures/conflicts of interest: Dr. Korde reports: Amgen Advisory Board and Genomic Health Speaker’s Bureau in the past. Dr. Stanczyk reports relationship with: Merck, Agile Therapeutics and TherapeuticsMD; No other disclosures reported.

Author Contributions: Study concept and design: McTiernan; *Acquisition of data:* Mason, Imayama, Duggan, Korde, McTiernan; *Analysis and interpretation of data:* Mason, Tapsoba, Stanczyk, McTiernan; *Drafting of the manuscript:* Mason; *Critical revision of the manuscript:* Duggan, Imayama, Wang, Korde, Stanczyk, McTiernan. CM had primary responsibility for final content. All authors read and approved the final manuscript.

D₃ or ii) weight loss + daily placebo. Serum sex hormone binding globulin, estrone, total, free and bioavailable estradiol and testosterone were measured by radioimmunoassay pre-randomization and at 12 months. Mean changes were compared between groups (intent-to-treat) using generalized estimating equations.

Results—12-month changes in sex hormone binding globulin, estrone, total, free and bioavailable estradiol and testosterone did not differ between groups (all $p>0.05$). However, a greater increase in serum 25-hydroxyvitamin D was associated with a greater increase in sex hormone binding globulin ($p_{\text{trend}}=0.01$), and larger decreases in free and bioavailable estradiol ($p_{\text{trend}}=0.04$, $p_{\text{trend}}=0.03$, respectively). In post-hoc analyses we compared women randomized to vitamin D whose serum 25-hydroxyvitamin D remained insufficient ($n=38$), to women who became replete (25-hydroxyvitamin D ≥ 32 ng/mL; $n=53$). Replete women showed greater reductions in bioavailable estradiol (-1.8 versus -0.7 pg/mL), free testosterone (-0.8 versus -0.3 pg/mL) and bioavailable testosterone (-1.8 versus -0.6 ng/dL), and a greater increase in sex hormone binding globulin (10.6 versus 4.7 nmol/L) (all $p<0.05$) even after adjusting for differences in total 12-month weight loss.

Conclusions—Overall, 12-month changes in sex hormone did not differ between groups. However, vitamin D repletion was associated with greater reductions in sex hormones during weight loss, with a possible dose-dependent effect. Future studies should test higher doses and target circulating 25-hydroxyvitamin D levels when measuring such effects.

Keywords

25-hydroxyvitamin D; obesity; estrogens; androgens

INTRODUCTION

Obesity is an established risk factor for postmenopausal breast cancer,^{1–3} although the underlying mechanisms have not been fully elucidated. Elevated sex hormone concentrations are hypothesized to play a role as adipose tissue is the primary peripheral site of aromatase production, and therefore, the primary site of sex steroid hormone synthesis after menopause.^{4–7} Vitamin D insufficiency is frequently observed with obesity;^{8, 9} low vitamin D status is also associated with postmenopausal breast cancer risk. A meta-analysis of nine prospective studies examining circulating vitamin D showed a non-linear inverse association between vitamin D and breast cancer risk, with diminishing benefit above 35 ng/mL¹⁰ in postmenopausal but not premenopausal women. In addition, in vitro and in vivo studies show that calcitriol, the hormonally active form of vitamin D, decreases aromatase expression and attenuates estrogen signaling in breast cancer cells.¹¹ Other proposed mechanisms through which vitamin D status may affect breast cancer risk include effects on cell division, apoptosis, and contact inhibition.¹²

The purpose of this study was to investigate the effects of 12 months of vitamin D₃ supplementation (2000 IU/day) versus placebo on changes in sex steroid hormones, including estradiol, estrone, testosterone, and sex hormone binding globulin (SHBG) among overweight and obese postmenopausal women with low circulating vitamin D (serum 25(OH)D $10 - <32$ ng/mL) participating in a lifestyle-based weight loss program. We

hypothesized that given excess sex hormones levels in overweight and obese postmenopausal women, vitamin D in conjunction with weight loss would provide additional sex hormone lowering effects compared to weight loss alone.

MATERIALS & METHODS

This study is ancillary to the Vitamin D, Diet and Activity (ViDA) study (www.clinicaltrials.gov Identifier NCT01240213), a 12-month double-blind, placebo-controlled randomized trial comparing the effects of 2000 IU/day oral Vitamin D₃ (cholecalciferol) supplementation versus placebo on weight and biomarkers of breast cancer risk, in postmenopausal women participating in a weight-loss program.¹³ Study procedures were reviewed and approved by the Fred Hutchinson Cancer Research Center Institutional Review Board. All participants provided written Informed Consent.

Study participants

The study is described elsewhere.¹³ Briefly, 218 postmenopausal (50–75 years) women with a body mass index (BMI) ≥ 25 kg/m² and serum 25(OH)D concentrations ≥ 10 ng/mL and <32 ng/mL ('insufficient') were randomly assigned to 12 months of either: i) 2000 IU/day vitamin D₃ supplementation + a lifestyle-based weight-loss program (n=109; 'Vitamin D'), or ii) daily placebo + a lifestyle-based weight-loss program (n=109; 'Placebo'). Exclusion criteria included: taking >400 IU supplemental vitamin D; renal disease; history of kidney stones; diagnosed diabetes, osteoporosis, or severe congestive heart failure; history of breast cancer or other invasive cancer excluding non-melanoma skin cancer; hormone replacement therapy use within the past 6 months; alcohol intake >2 drinks/day; current smoking; contraindication to taking 2000IU vitamin D₃/day; history of bariatric surgery; use of weight loss medications; additional factors that might interfere with the measurement of outcomes or intervention success (e.g., inability to attend facility-based sessions).

Women were recruited primarily through mass-mailings and media publicity. Invitation letters (n=6,253) were mailed to potentially eligible women identified from a database of previous study participants and women who had expressed an interest in our studies. In total, 2,147 women returned an interest survey, 498 of whom met initial eligibility criteria and were invited for vitamin D screening. Of these, 310 were eligible based on study 25-hydroxyvitamin D criteria (≥ 10.0 ng/mL and <32.0 ng/mL). A total of 264 women underwent a clinic screening interview and 218 women were randomized into the study (Figure 1).¹³

Randomization and interventions

Participants were randomized by permuted blocks randomization (1:1), stratified according to BMI (<30 or ≥ 30 kg/m²) and whether consent was given for optional breast and abdominal fat biopsies. All staff except statisticians were blinded to randomization status. The number of women randomized to each arm did not differ by season ($p>0.99$).

The vitamin D and matching placebo (sunflower oil) gel capsules were created and bottled in unmarked containers by J.R. Carlson Laboratories, Inc. (Arlington, IL, USA). At randomization, participants received a 6-month supply of study medications. Medication

bottles were returned at 6 months and remaining capsules were counted before a second 6 month supply was provided. Likewise, at 12 months, the second bottle and any remaining capsules were returned and counted. Ten percent of study medication bottles were randomly chosen for quality assurance at 6 months and 12 months. One hundred percent of the tested capsules matched the assigned content provided by the lab. Medication counts and the 12-month change in serum vitamin D were used as indicators of study medication adherence.

The ViDA weight loss program, administered to all participants, included both a diet and exercise component adapted from a successful intervention originally based on the Diabetes Prevention Program and Look Ahead weight loss programs.^{14,15} and previously used by our group in a similar study population.^{16,14, 15} The goals of the program were total daily energy intake of 1200–2000 kcal/day based on baseline weight, <30% daily energy intake from fat, 45 minutes of moderate-to-vigorous intensity exercise 5 days/week, and a 10% reduction in body weight. Exercise sessions were performed in our supervised facility and at home.¹³ Diets were not supplemented with calcium, but women were advised on how to obtain sufficient calcium in their diets.

Women receiving vitamin D₃ attended a mean 56.1% of all diet counseling sessions and completed a mean (SD) 138 (147) mins/wk of moderate-to-vigorous physical activity while women in the placebo arm attended a mean 59.5% of diet sessions and completed a mean (SD) 147 (140) mins/wk of activity. The 12-month change in vitamin D intake from dietary sources and supplements did not differ between study arms ($p=0.60$). Both groups increased their average pedometer steps/day (+2602 and +2816 steps/d, respectively) compared to baseline. No significant differences in adherence to the weight loss program were detected between study arms.

Outcome measures

Prior to randomization and at 12 months, participants provided a 50 mL blood sample after fasting for 12 hours, not exercising for 24 hours, or drinking alcohol for 48 hours. Blood was processed within 1 hour and serum stored at -70°C until analysis.

Vitamin D was measured at Heartland Assays, Inc (Ames, Iowa, USA) with a direct, competitive chemiluminescence immunoassay (CLIA) using the DiaSorin LIAISON 25-OH Vitamin D Total assay which is co-specific for 25-hydroxyvitamin D₃ and 25-hydroxyvitamin D₂ (29, 30). The inter- and intra-assay CVs for this assay were 11.2 % and 8.1% respectively.

All other laboratory assays were performed at the Reproductive Endocrine Research Laboratory (University of Southern California, Los Angeles, CA, USA). Estrone, estradiol, and total testosterone were quantified by specific radioimmunoassays after organic solvent extraction and Celite column partition chromatography.^{17, 18} SHBG was measured with a chemiluminescent immunoassay using the Immulite analyzer (Siemens Medical Solutions Diagnostics, Malvern, PA, USA) to allow calculation of bioavailable (free plus albumin-bound) estradiol and testosterone.¹⁹

Baseline and 12-month samples from each individual were included in the same batch, and participants' samples were randomly placed across batches. Serum 25(OH)D was measured as previously described.¹³ Interassay coefficients of variation (CV) ranged from 5–18% for the sex steroid hormone assays; the interassay CV for the SHBG assay was 6%.

Covariates

Demographic information, medical history, anthropometric measures, and lifestyle behaviors, including sun exposure, physical activity, dietary intake and supplement use were collected as previously described.¹³

Safety and adverse events—All participants were interviewed after 1, 3, 6, 9 and 12 months of study participation for any signs or symptoms of vitamin D toxicity or other adverse events, including serious illness or hospitalizations. Reports were reviewed by a physician's assistant with appropriate follow-up as necessary. Summary data were recorded and reviewed according to study group by an independent Data and Safety Monitoring Committee at 6-month intervals.

Statistical analyses

Assuming 80% power in this sample, we calculated minimum detectable absolute differences between study arms of 8–14% for our outcomes.

Age-adjusted partial correlation coefficients were calculated between baseline measures. Outcome measures were log-transformed to improve distribution normality and are presented as geometric means $\pm$ 95% confidence interval (CI) unless otherwise noted. Mean 12-month changes between groups were compared using the generalized estimating equations (GEE) modification of linear regression to account for intra-individual correlation over time. Models were initially unadjusted, and subsequently adjusted for age, race/ethnicity (white, other), baseline 25(OH)D, baseline BMI, total vitamin D intake (diet + non-study supplements), average sun exposure (hrs/day), and total 12-month weight loss. The GEE approach for mixed-model regression using available data was applied to address missing data.

Additional analyses were based on post-hoc analyses of specific subgroups. Outcomes were compared across tertiles of change in serum 25(OH)D, according to whether or not women achieved our pre-study definition of repletion (25(OH)D $\geq$ 32 ng/mL), in women with versus without complete pill counts, and across categories of weight loss (no change/gained (referent); lost $<$ 5% baseline weight; lost 5– $<$ 10% baseline weight; lost $\geq$ 10% baseline weight).¹⁶

All statistical tests were two-sided. Statistical analyses were performed using SAS software (version 9.3, SAS Institute Inc., Cary, NC).

RESULTS

The baseline characteristics of randomized women are shown in Table 1.¹³ The majority (86%) were non-Hispanic white. The mean age and BMI were 59.6 ± 5.1 years and 32.4

$\pm 5.8 \text{ kg/m}^2$, respectively. The mean baseline serum 25(OH)D concentration was $21.4 \pm 5.1 \text{ ng/mL}$. At 12 months, 187 (86%) women provided a blood sample; however, only 55% remembered to return their unused study pills. Thus, complete pill counts are available only for a subset of participants (Vit D: 54%, Placebo: 56%) but compliance was high among them (Vit D: 98%, Placebo: 96%). Six women were excluded from analyses because of serum estradiol values outside of acceptable postmenopausal ranges ($>42 \text{ pg/mL}$).²⁰

Serum 25(OH)D increased a mean of 13.6 ng/mL in the vitamin D arm and decreased a mean of 1.3 ng/mL in the placebo arm over 12 months ($p < 0.0001$). The mean weight change was -8.2% in the vitamin D arm compared to -8.4% in the placebo arm ($p = 0.41$).¹³

At baseline, serum 25(OH)D was positively associated with total vitamin D intake ($r = 0.20$, $p = 0.02$), but not with serum estrone ($r = -0.07$, $p = 0.33$), total, free, or bioavailable estradiol (all $r = -0.1$, $p > 0.05$), or SHBG ($r = 0.004$, $p = 0.95$). Serum 25(OH)D was significantly inversely associated with total, free, and bioavailable testosterone (all $r = -0.15$, $p = 0.03$).

Although sex hormone concentrations were reduced in both groups, the mean magnitude of change over 12 months was not significantly different between the vitamin D and placebo arms for any outcome measure (all $p > 0.05$; Table 2). However, when stratified according to magnitude of change in 25(OH)D using tertiles, greater increases in serum 25(OH)D were associated with greater increases in SHBG in a dose-dependent manner ($p_{\text{trend}} = 0.01$), and with greater decreases in free and bioavailable estradiol ($p_{\text{trend}} = 0.04$, $p_{\text{trend}} = 0.03$, respectively) after adjusting for age, race/ethnicity, baseline BMI, baseline serum 25(OH)D, vitamin D intake (diet + non-study supplements), average sun exposure, and total 12-month weight loss (Table 3).

Compared to women randomized to the vitamin D arm who did not become replete despite supplementation ($N = 38$), women who became replete (i.e., 25(OH)D $\geq 32 \text{ ng/mL}$; $N = 53$) by 12 months showed a greater reduction in free estradiol (-0.07 versus -0.03 pg/mL , $p = 0.03$) and bioavailable estradiol (-1.8 versus -0.7 pg/mL , $p = 0.02$), greater reductions in free testosterone (-0.8 versus -0.3 pg/mL , $p = 0.003$) and bioavailable testosterone (-1.8 versus -0.6 ng/dL , $p = 0.003$), and a greater increase in SHBG (10.6 versus 4.7 nmol/L , $p = 0.005$) after adjusting for age, race/ethnicity, baseline BMI, baseline 25(OH)D, total vitamin D intake, and average sun exposure. Further adjustment for total 12-month weight loss attenuated these associations but most remained statistically significant (Table 4).

In analyses limited to women with complete study pill counts, SHBG increased to a larger degree in the vitamin D arm compared to placebo (Vit D: 11.64 nmol/L (27.9%) versus P: 6.35 nmol/L (14.9%), $p = 0.03$), while free estradiol (Vit D: -0.07 pg/mL (-23.9%) versus P: -0.04 pg/mL (-13.4%), $p = 0.02$) and bioavailable estradiol (Vit D: -1.73 pg/mL (-23.9%) versus P: -0.99 pg/mL (-13.8%), $p = 0.02$) declined more among women taking vitamin D compared to placebo after adjusting for all of the covariates listed above (Table 5). No effect modification by degree of weight loss was detected (results not shown).

DISCUSSION

Over two-thirds of American women are overweight or obese²¹, and approximately 75% have vitamin D levels below 30 ng/mL which is generally considered insufficient.²² Although our results suggest no overall benefit of 2000 IU/day vitamin D supplementation on reducing sex hormone concentrations beyond the effect of weight loss alone in postmenopausal women, a greater increase in 25(OH)D was significantly associated with decreases in free and bioavailable estradiol, and increases in SHBG. Moreover, repletion to levels ≥ 32 ng/mL was associated with significantly greater rises in SHBG and significant reductions in bioavailable estradiol, free and bioavailable testosterone, compared to women whose circulating 25(OH)D remained below 32 ng/mL; these effects were independent of total weight loss.

In analyses limited to women with complete pill counts, among whom medication adherence was 97% and changes in 25(OH)D were +15.7 ng/mL and -1.1 ng/mL in the vitamin D and placebo arms, respectively; estradiol decreased, and SHBG increased significantly more in the vitamin D arm compared to placebo. Although a significant mean increase in serum 25(OH)D was still observed in the vitamin D arm compared to placebo (10.1 versus -1.8 ng/mL, $p < 0.001$) among women without complete pill counts, lower compliance in a sub-set of study participants may have diminished the overall strength of the observed outcomes.

Weight loss through caloric restriction and exercise has been shown to significantly reduce biomarkers of postmenopausal breast cancer risk;²⁰ however, to our knowledge, no other studies have examined the effects of vitamin D supplementation on sex steroid hormones during weight loss. It remains unknown whether vitamin D-related outcomes are more strongly related to a specific magnitude of change in 25(OH)D (e.g. 10 ng/mL) or to a change in status defined by reaching a specific threshold level (e.g. ≥ 32 ng/mL). This remains an important area for future investigation, as does the effect of individualizing vitamin D therapy to repletion at specific levels.

Strengths of our study include its double-blind randomized controlled design and its relatively long duration. However, all significant results were based on post-hoc analyses of subgroups; thus, our results should be considered hypothesis-generating for future studies. There remains no consensus definition for vitamin deficiency and insufficiency^{23, 24} and we may have observed stronger effects using a more stringent definition of insufficiency in our study sample. For example, we excluded women with serum 25(OH)D concentrations < 10 ng/mL, among whom the effect of vitamin D supplementation could be more pronounced. Additionally, we tested only one dose of supplementation, had complete study medication counts for only 55% of participants, and did not test the independent effects of vitamin D without a weight loss intervention. Finally, because our study population was relatively homogeneous our results may not be generalizable to other racial/ethnic groups.

CONCLUSIONS

The relationships between vitamin D and sex steroid hormones warrant further investigation in carefully designed studies to more fully elucidate its potential role in the prevention of postmenopausal breast cancer.

Acknowledgments

Financial Support:

Supported by grants from the Breast Cancer Research Foundation, Susan G. Komen for the Cure Scientific Advisory Council Award 2010–12, and the National Cancer Institute (NCI) R03 CA162482-01.

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Figure 1.
Flow pf participants through the Vitamin D, Diet and Exercise (ViDA) study.

Table 1Selected baseline characteristics of ViDA study participants^a

Variable	N (%) or Mean (SD)		
	All	Placebo	Vitamin D
Age (years)	59.6 (5.1)	59.0 (4.7)	60.3 (5.3)
Weight (kg)	87.7 (16.3)	88.1 (17.1)	87.4 (15.5)
BMI (kg/m ²)	32.4 (5.8)	32.5 (6.1)	32.3 (5.5)
Body Fat (%)	47.4 (4.9)	47.5 (4.5)	47.3 (5.2)
Waist circumference (cm)	100.1 (12.3)	100.3 (13.5)	100.0 (11.0)
Race/Ethnicity [n, (%)]			
Non-Hispanic White	188 (86.2%)	94 (86.2)	94 (86.2)
Non-Hispanic Black	13 (6.0%)	6 (5.5)	7 (6.4)
Hispanic	5 (2.3%)	4 (3.7)	1 (0.9)
Other (American Indian, Asian or Unknown)	12 (5.5%)	5 (4.6)	7 (6.4)
College graduate [n, (%)]	161 (73.9%)	79 (72.5)	82 (75.2)
Moderate to vigorous physical activity (min/wk)	142.2 (143.2)	146.6 (140.4)	137.9 (146.5)
Energy intake (kcal/d) ^b	2004 (699.3)	1982 (678)	2025 (722)
Relative % energy from fat	33.0 (6.2)	32.6 (5.7)	33.4 (6.7)
Relative % energy from protein	17.6 (3.2)	17.9 (3.5)	17.2 (2.9)
Relative % energy from carbohydrate	48.3 (7.4)	48.1 (7.1)	48.5 (7.8)
Dietary vitamin D intake (µg)	6.6 (4.6)	6.9 (5.2)	6.3 (4.0)
Vitamin D supplement intake (IU)	280.0 (134.5)	303.6 (125.2)	262.7 (140.5)
Total calcium intake, diet + supplement (mg)	1120 (600)	1170 (633)	1071 (564)
Sun exposure (hrs/wk) ^c	2.4 (1.3)	2.2 (1.3)	2.5 (1.3)
Serum 25(OH)D (ng/mL)	21.4 (6.1)	21.4 (6.1)	21.4 (6.2)
Estrone (pg/mL)	43.3 (17.3)	43.3 (17.4)	43.2 (17.3)
Estradiol (pg/mL)	12.7 (6.0)	12.5 (5.7)	13.0 (6.2)
Free Estradiol (pg/mL)	0.33 (0.18)	0.33 (0.18)	0.34 (0.19)
Bioavailable Estradiol (pg/mL)	8.5 (4.6)	8.4 (4.5)	8.6 (4.7)
Testosterone (ng/dL)	25.6 (12.0)	24.4 (10.7)	26.7 (13.0)
Free Testosterone (ng/dL)	5.2 (2.5)	5.0 (2.4)	5.3 (2.6)
Bioavailable Testosterone (ng/dL)	12.6 (6.2)	12.3 (6.0)	13.0 (6.5)
Sex hormone binding globulin (nmol/L)	46.1 (22.4)	45.1 (21.8)	47.0 (22.9)

^aSex hormone concentrations exclude six participants with estradiol >42pg/mL^bValues derived from FFQ were truncated <600 kcal and >4000 kcal.^cCalculated based on average exposure between 1000 and 1600; reported separately for weekdays and weekends.

Table 2

12-month change in sex hormone concentrations by study arm.

	PLACEBO				VITAMIN D			
	Geo Mean (95% CI)		Change	%	Geo Mean (95% CI)		Change	%
	Baseline	12 months			Baseline	12 months		
Estrone (pg/mL)	40.3 (37.5, 43.4)	37.9 (35.1, 41.0)	-2.4	-6.0	40.3 (37.6, 43.2)	37.0 (34.4, 43.8)	-3.3	-8.1
Estradiol (pg/mL)	11.4 (10.5, 12.3)	10.2 (9.3, 11.2)	-1.2	-10.2	11.8 (10.9, 12.8)	10.1 (9.2, 11.0)	-1.7	-14.4
Free Estradiol (pg/mL)	0.29 (0.26, 0.32)	0.25 (0.22, 0.28)	-0.04	-14.6	0.30 (0.27, 0.33)	0.24 (0.22, 0.26)	-0.06	-19.5
Bioavailable Estradiol (pg/mL)	7.4 (6.7, 8.1)	6.3 (5.6, 7.1)	-1.1	-14.7	7.6 (6.9, 8.3)	6.1 (5.5, 6.7)	-1.5	-19.6
Testosterone (ng/dL)	22.1 (20.2, 24.1)	21.6 (19.5, 23.9)	-0.5	-2.3	24.1 (22.1, 26.2)	23.0 (20.7, 25.6)	-1.1	-4.4
Free Testosterone (pg/mL)	4.4 (4.0, 4.9)	4.0 (3.6, 4.5)	-0.42	-9.5	4.7 (4.3, 5.2)	4.1 (3.7, 4.6)	-0.6	-12.8
Bioavailable Testosterone (ng/dL)	10.9 (9.9, 12.0)	9.9 (8.8, 11.0)	-1.0	-9.3	11.6 (10.6, 12.7)	10.2 (9.1, 11.3)	-1.5	-12.6
Sex hormone binding globulin (nmol/L)	40.6 (37.2, 44.4)	47.1 (42.9, 51.7)	6.5	16.0	42.1 (38.5, 46.1)	50.3 (46.0, 55.1)	8.2	19.5

^a GEE model comparing the 12-mo change between vitamin D vs. placebo; all available data, unadjusted.^b Adjusted for age, race/ethnicity, baseline serum 25(OH)D, and baseline BMI.^c Adjusted for age, race/ethnicity, baseline serum 25(OH)D, baseline BMI, vitamin D intake (diet + non-study supplements), average sun exposure (hrs/d), and total 12-month weight loss

12-month log-transformed change in sex hormones, stratified by tertiles^a of change in serum 25(OH)D among women receiving vitamin D (2000 IU/day).

Table 3

	Baseline			12 months			Change	<i>p^b</i>	<i>p^c</i>	<i>p^d</i>
	GeoMean	95% CI	GeoMean	95% CI	GeoMean	95% CI				
Estrone (pg/mL)										
Placebo	40.3	37.5, 43.4	37.9	35.1, 41.0			-2.4			
T1	41.2	36.0, 47.1	38.1	32.8, 44.2			-3.1	<i>ref</i>	<i>ref</i>	<i>ref</i>
T2	36.2	31.5, 41.5	35.5	31.5, 39.9			-0.7	0.32	0.89	0.51
T3	42.0	37.0, 47.6	37.5	33.6, 42.0			-4.5	0.48	0.29	0.66
Ptrend							0.47	0.26	0.62	
Estradiol (pg/mL)										
Placebo	11.4	10.5, 12.3	10.2	9.3, 11.2			-1.2			
T1	11.5	9.8, 13.6	10.6	8.9, 12.6			-0.9	<i>ref</i>	<i>ref</i>	<i>ref</i>
T2	10.5	9.1, 12.2	9.5	8.2, 10.9			-1.1	0.75	0.68	0.82
T3	12.6	11.0, 14.4	10.2	8.9, 11.6			-2.4	0.02	0.02	0.14
Ptrend							0.02	0.02	0.13	
Free Estradiol (pg/mL)										
Placebo	0.29	0.26, 0.32	0.25	0.22, 0.28			-0.04			
T1	0.30	0.25, 0.35	0.26	0.22, 0.32			-0.03	<i>ref</i>	<i>ref</i>	<i>ref</i>
T2	0.26	0.22, 0.31	0.22	0.19, 0.27			-0.04	0.62	0.69	0.66
T3	0.32	0.27, 0.38	0.23	0.20, 0.27			-0.09	0.001	0.005	0.04
Ptrend							0.001	0.004	0.04	
Bioavailable Estradiol (pg/mL)										
Placebo	7.4	6.7, 8.1	6.3	5.6, 7.1			-1.10			
T1	7.5	6.3, 9.0	6.7	5.6, 8.1			-0.8	<i>ref</i>	<i>ref</i>	<i>ref</i>
T2	6.6	5.5, 7.9	5.6	4.7, 6.8			-0.9	0.60	0.65	0.74
T3	8.2	7.0, 9.6	5.9	5.1, 6.9			-2.2	<0.001	0.004	0.03
Ptrend							<0.001	0.003	0.03	
Testosterone (ng/dL)										
Placebo	22.1	20.1, 24.1	21.6	19.5, 23.9			-0.5			

	Baseline		12 months			Change	pb	pc	pd
	GeoMean	95% CI	GeoMean	95% CI					
Free Testosterone (pg/mL)									
T1	24.6	24.6, 20.6	22.7	18.3, 28.1	-1.9	ref	ref	ref	
T2	23.8	23.8, 20.7	23.4	19.9, 27.6	-0.3	0.32	0.49	0.74	
T3	23.2	23.2, 19.6	22.9	19.2, 27.3	-0.3	0.29	0.67	0.97	
Ptrend						0.29	0.74	0.99	
Bioavailable Testosterone (ng/dL)									
Placebo	4.4	4.0, 4.9	4.0	3.6, 4.5	-0.4				
T1	5.0	4.2, 5.9	4.4	3.6, 5.4	-0.6	ref	ref	ref	
T2	4.5	3.9, 5.2	4.1	3.5, 4.9	-0.4	0.56	0.54	0.91	
T3	4.6	3.9, 5.6	3.9	3.2, 4.7	-0.7	0.43	0.07	0.37	
Ptrend						0.43	0.07	0.35	
SHBG (nmol/L)									
Placebo	10.9	9.9, 12.0	9.9	8.8, 11.0	-1.000				
T1	12.2	10.3, 14.5	10.8	8.8, 13.2	-1.4	ref	ref	ref	
T2	11	9.5, 12.8	10.1	8.5, 12.1	-0.9	0.57	0.52	0.89	
T3	11.4	9.5, 13.6	9.6	7.9, 11.7	-1.8	0.45	0.07	0.38	
Ptrend						0.45	0.07	0.37	
SHBG (nmol/L)									
Placebo	40.6	37.2, 44.4	47.1	42.9, 51.7	6.5				
T1	40.5	34.8, 47.1	44.1	38.0, 51.3	3.7	ref	ref	ref	
T2	45.6	38.2, 54.4	52.1	44.1, 61.6	6.6	0.42	0.81	0.74	
T3	40.9	34.6, 48.3	55.3	47.7, 64.2	14.4	0.004	0.009	0.02	
Ptrend						0.004	0.006	0.01	

^a Tertile 1 (T1): n=31, 25(OH)D <9.43 ng/mL; Tertile 2 (T2): n=31, 25(OH)D 9.43–16.65 ng/mL; Tertile 3 (T3): n=31, 25(OH)D ≥16.65 ng/mL.

^b p= Compared to lowest tertile of 25(OH)D, unadjusted;

^c p= Compared to lowest tertile of 25(OH)D, adjusted for age, race-ethnicity, baseline 25(OH)D, and baseline BMI.

^d p= Compared to lowest tertile of 25(OH)D, adjusted for age, race-ethnicity, baseline 25(OH)D, baseline BMI, vitamin D intake (diet +non study supplements), average sun exposure (hrs/d), and total 12-mo weight loss.

Change in sex hormones among women randomized to 2000IU vitamin D/day who did vs. did not become replete (25(OH)D 32ng/mL) by 12 months.

Table 4

	25(OH)D <32ng/mL (n=38)				25(OH)D 32ng/mL (n=53)			
	Mean (SD) or Geo Mean (95% CI)		Change	%	Mean (SD) or Geo Mean (95% CI)		Change	%
	Baseline	12 months			Baseline	12 months		
Weight (kg)	89.7 (16.6)	84.1 (16.7)	-5.6	-6.3	85.7 (14.2)	77.2 (14.3)	-8.5	-9.9
Estrone (pg/mL)	38.4 (33.6, 43.7)	37.3 (32.8, 42.3)	-1.1	-2.9	40.7 (37.1, 44.7)	36.8 (33.8, 40.2)	-3.9	-9.5
Estradiol (pg/mL)	11.2 (9.8, 12.8)	10.5 (9.1, 12.1)	-0.7	-6.3	11.8 (10.5, 13.2)	9.8 (8.8, 10.9)	-2.0	-16.8
Free Estradiol (pg/mL)	0.28 (0.24, 0.33)	0.25 (0.22, 0.30)	-0.03	-9.7	0.30 (0.26, 0.34)	0.23 (0.20, 0.26)	-0.07	-23.1
Bioavailable Estradiol (pg/mL)	7.1 (6.1, 8.3)	6.4 (5.5, 7.6)	-0.7	-9.4	7.6 (6.7, 8.7)	6.1 (5.5, 6.7)	-1.8	-23.5
Testosterone (ng/dL)	24.7 (21.2, 28.8)	24.6 (20.6, 29.3)	-0.1	-0.5	23.2 (20.7, 26.0)	21.9 (19.2, 25.0)	-1.3	-5.5
Free Testosterone (pg/mL)	4.8 (4.1, 5.6)	4.6 (3.8, 5.4)	-0.3	-5.4	4.6 (4.1, 5.2)	3.9 (3.4, 4.4)	-0.8	-16.3
Bioavailable Testosterone (ng/dL)	11.8 (10.1, 13.8)	11.2 (9.4, 13.3)	-0.6	-5.3	11.3 (10.0, 12.8)	9.5 (8.3, 10.9)	-1.8	-16.1
Sex hormone binding globulin (nmol/L)	43.2 (37.2, 50.2)	48.0 (41.9, 54.9)	4.7	11.0	41.5 (36.7, 47.0)	52.1 (46.1, 58.9)	10.6	25.5

^a GEE model comparing the 12-mo change between vitamin D vs. placebo; all available data, unadjusted.

^b Adjusted for age, race/ethnicity, baseline serum 25(OH)D, baseline BMI, total vitamin D intake (diet + non-study supplements), average sun exposure (hrs/d).

^c Adjusted for age, race/ethnicity, baseline serum 25(OH)D, baseline BMI, total vitamin D intake, average sun exposure, and total 12-month weight change.

Table 5

12-month change [SE] in sex hormones in women randomized to 2000 IU vitamin D/day or placebo: women with complete pill counts [N=120].

	12-Month Change (Mean [SE])		
	Placebo [N=61]	Vitamin D [N=59]	
Estrone [pg/mL]	-3.15 [1.04]	-3.87 [1.06]	<i>p^a</i> 0.50 <i>p^b</i> 0.39 <i>p^c</i> 0.32
Estradiol [pg/mL]	-1.04 [0.33]	-1.91 [0.32]	0.04 0.04 0.07
Free Estradiol [pg/mL]	-0.04 [0.01]	-0.07 [0.01]	0.008 0.01 0.02
Bioavailable Estradiol [pg/mL]	-0.99 [0.24]	-1.73 [0.24]	0.01 0.01 0.02
Testosterone [ng/dL]	-1.25 [0.55]	-1.23 [0.72]	0.95 0.48 0.72
Free Testosterone [pg/mL]	-0.55 [0.12]	-0.75 [0.13]	0.15 0.28 0.29
Bioavailable Testosterone [ng/dL]	-1.31 [0.29]	-1.83 [0.33]	0.14 0.23 0.25
SHBG [nmol/L]	6.35 [0.17]	11.64 [1.61]	0.009 0.005 0.03

^a GEE model comparing the 12-mo change between vitamin D vs. placebo; all available data, unadjusted.

^b Adjusted for age, race/ethnicity, baseline serum 25[OH]D and baseline BMI.

^c Adjusted for age, race/ethnicity, baseline serum 25[OH]D, baseline BMI, vitamin D intake [diet + non-study supplements], average sun exposure [hrs/d], and total 12-month weight loss.