Vitamin D_3 Supplementation Has No Effect on Conventional Cardiovascular Risk Factors: A Parallel-Group, Double-Blind, Placebo-Controlled RCT

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Context: Observational studies show an association between low vitamin D status assessed by circulating 25-hydroxyvitamin D and cardiovascular events and mortality. Data from randomized controlled trials are limited.

Objective: The aim of this study was to test whether daily doses of vitamin D_3 at 400 or 1000 IU/d for 1 yr affected conventional markers of cardiovascular disease (CVD) risk.

Design: We conducted a parallel-group, double-blind, placebo-controlled randomized controlled trial. Randomization was computer generated. Participants and study investigators were blinded to intervention groupings throughout the trial.

Setting: The study was conducted at the Clinical Research Facility, University of Aberdeen, United Kingdom.

Participants: A total of 305 healthy postmenopausal women aged 60–70 yr were recruited for the study.

Intervention: Each woman received a daily capsule of 400 or 1000 IU vitamin D_3 or placebo randomly allocated.

Main Outcome Measures: Primary outcomes were serum lipid profile [total, high-density lipoprotein, and low-density lipoprotein cholesterol; triglycerides; and apolipoproteins A-1 and B_{100}], insulin resistance (homeostatic model assessment), inflammatory biomarkers (high-sensitivity C-reactive protein, IL-6, soluble intracellular adhesion molecule-1), and blood pressure.

Results: A total of 265 (87%) participants completed all study visits. Small differences between groups for serum apolipoprotein B_{100} change [repeated measures ANOVA, $P = 0.04$; mean (SD), -1.0 (10.0) mg/dl (400 IU); -1.0 (10.0) mg/dl (1000 IU); and $+0.02$ (10.0) mg/dl (placebo)] were not considered clinically significant. Other systemic markers for CVD risk remained unchanged. There was significant seasonal variation in systolic and diastolic blood pressure independent of vitamin D dose ($P < 0.001$, linear mixed model). Mean (SD) reduction in systolic blood pressure from winter to summer was -6.6 (10.8) mm Hg.

Conclusions: Improving vitamin D status through dietary supplementation is unlikely to reduce CVD risk factors. Confounding of seasonality should be recognized and addressed in future studies of vitamin D. **(*J Clin Endocrinol Metab* 97: 3557–3568, 2012)**

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Abbreviations: APO, Apolipoprotein; CI, confidence interval; CVD, cardiovascular disease; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; 25(OH)D, 25-hydroxyvitamin D; RCT, randomized controlled trial; SED, standard erythemal dose; sICAM-1, soluble intracellular adhesion molecule-1.

Multiple observational studies have consistently shown an association between low vitamin D status assessed by circulating total 25-hydroxyvitamin D [25(OH)D] and cardiovascular events, cardiovascular mortality, and cerebrovascular mortality (1–8). Concentrations of total 25(OH)D below 25–37.5 nmol/liter have been associated with the greatest risk of cardiovascular disease (CVD) incidence and mortality in prospective study populations (8). Several mechanisms suggest a protective role for vitamin D in CVD development. 1,25-Dihydroxyvitamin D, the active form of the vitamin, inhibits vascular smooth muscle cell proliferation (9) and vascular calcification (10), negatively regulates the renin-angiotensin-aldosterone system (11), suppresses inflammatory processes via powerful immunomodulatory effects (12, 13), and improves insulin secretion and sensitivity (14).

There is speculation on the potential for over-the-counter supplements containing vitamin D_2 or vitamin D_3 in the prevention or treatment of CVD (15). Randomized trials of vitamin D (many of which combine calcium supplements and were designed primarily to investigate musculoskeletal outcomes) have reported positive effects on a range of clinical markers of CVD risk such as blood pressure (1, 2, 16–18), inflammatory cytokines (2), and serum lipids (2), whereas others have reported little or no improvement (1, 2, 19, 20). These studies show marked variation in size, duration, participant population, primary outcomes, dosing regimen, intervention formulations, use of concomitant therapies, and quality of study design. A systematic review identified two supplementation trials with vitamin D only (one employing vitamin D_2 over 1 yr and the other vitamin D_3 over 5 yr at approximate doses of 1000 IU/d) reporting on CVD events as a secondary outcome (1). These studies found no reduction in risk for CVD events [pooled relative risk, 0.90; 95% confidence interval (CI), 0.77–1.05] with vitamin D supplementation.

Against this background, we performed a parallel-group, double-blind, placebo-controlled, randomized controlled trial (RCT). Study visits were fixed at 2-month intervals over 1 yr, with all participants starting the intervention at the beginning of the year to capture any potential seasonal effects. Our primary objective was to test whether daily doses of vitamin D_3 at 400 IU (UK reference nutrient intake for >65 yr and younger adults at risk of deficiency) or 1000 IU (UK safe upper limit at the time of study design) affected conventional markers of CVD risk in postmenopausal women.

Subjects and Methods

Study design and participants

Study visits were conducted at the Clinical Research Facility, University of Aberdeen, UK (57° N). Participants attended a study screening visit approximately 2 months before enrollment to assess their suitability for inclusion. Baseline visits took place between January and March 2009 and subsequently at 2-month intervals, with final visits between January and March 2010.

Participants [n = 305; age (SD), 63.8 (2.2) yr] were Caucasian postmenopausal women recruited between July and December 2008 from the Aberdeen Prospective Osteoporosis Screening cohort (21), selected randomly from Community Health Index records. Individuals with preexisting CVD, diabetes, asthma, malabsorption, hypertensive blood pressure measurements of at least 160 mm Hg systolic or 99 mm Hg diastolic, difficulty in swallowing tablets or capsules, or who were taking medications or supplements known to affect any dependent variable were excluded, as were current smokers or participants with abnormal blood biochemistry at screening. A summary of recruitment details is shown in Fig. 1. Participants gave written informed consent. Ethical approval was obtained from the Grampian Research Ethics Committee (08/S0802/73).

Interventions

Capsules containing vitamin D_3 (400 or 1000 IU) or identical placebo were purchased (Pure Encapsulations, Sudbury, MA), packaged into white plastic coded containers, and sealed in sequentially numbered study packs (Bilcare, Powys, UK). Research nurses assigned participants to one of three intervention groups using an automated telephone service (Health Services Research Unit, University of Aberdeen, UK): low-dose vitamin D_3 (400 IU/d), high-dose vitamin D_3 (1000 IU/d), and placebo. Body mass index category (<18.5, 18.5–24.99, 25–29.99, 30–39.99, or ≥40 kg/m^2) was employed as a minimization criterion. Participants were supplied with 2 months worth of study capsules (n = 65) at each visit and instructed to take one every day with breakfast. Capsules were independently analyzed after the code break (Eurofins Laboratories Ltd., West Midlands, UK). Both participants and study investigators were blinded to intervention groupings throughout the study. Participants were instructed not to take any dietary supplements containing vitamin D (including cod liver oil) throughout the duration of the study.

Primary study outcomes

Primary study outcomes were serum lipid profile, estimate of insulin resistance, inflammatory biomarkers, and blood pressure.

Serum lipid profile

Total and high-density lipoprotein (HDL) cholesterol, triglycerides, and apolipoprotein (APO) A-1 and APO B_{100} concentrations were analyzed using standard automated procedures (Clinical Biochemistry, Aberdeen Royal Infirmary, UK). Concentrations of total and HDL cholesterol and triglycerides were measured using the ADVIA 2400 Chemistry System (Siemens, Surrey, UK). Low-density lipoprotein (LDL) cholesterol was calculated (using the Friedwald formula), and concentrations of APO A-1 and APO B_{100} were measured using the ADVIA 1800 Chemistry System (Siemens).

Insulin resistance

Serum insulin concentrations were determined by immunoassay (IMMULITE 2500 Immunoassay System, Siemens), and glucose concentrations were measured by automated assay (AVIDA 1800 Chemistry System). Estimates of insulin

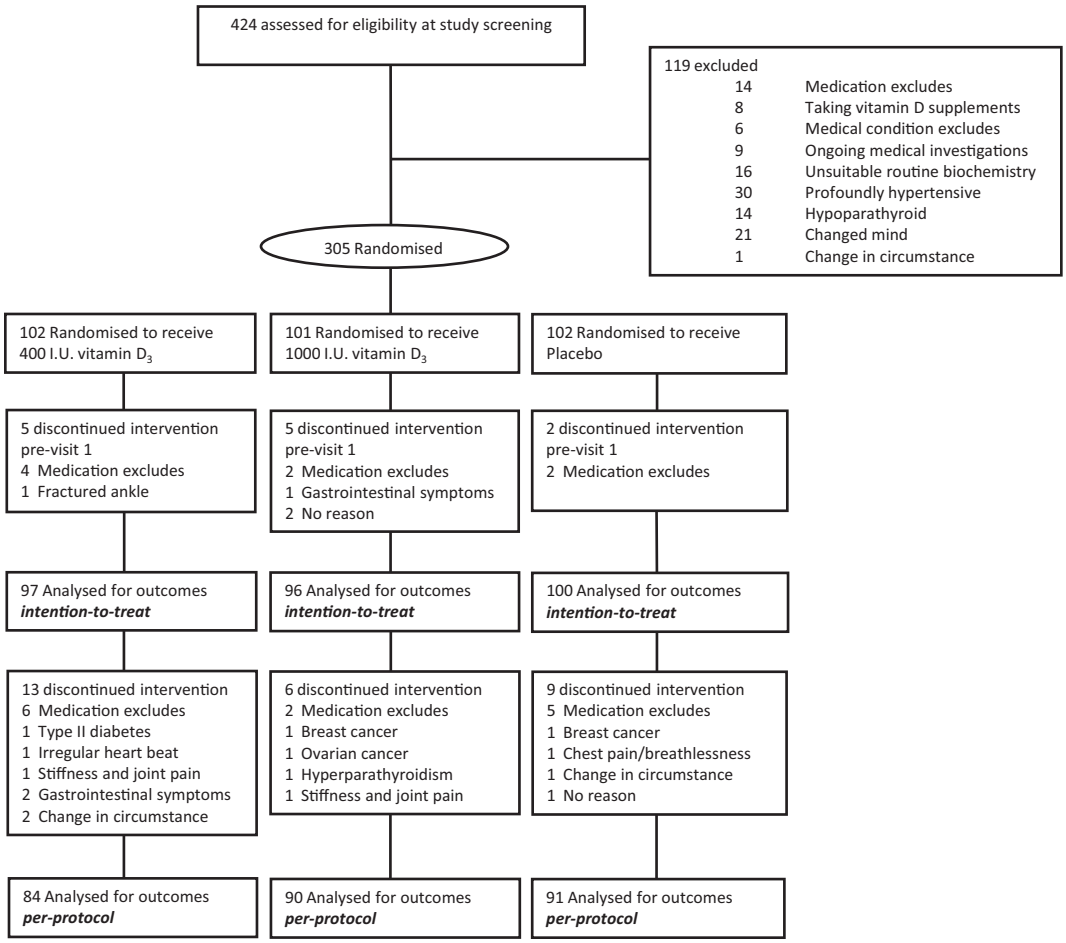

FIG. 1. Flow diagram of the study, showing numbers of participants who were randomly assigned, received the intended treatment, and were analyzed for the primary outcomes.

resistance were calculated using the homeostatic model assessment (HOMA-IR) (22).

Inflammatory markers

Serum concentrations of high-sensitivity C-reactive protein (hsCRP) were measured by automated assay (ADIVA 1800 Chemistry System), whereas IL-6 and soluble intracellular adhesion molecule-1 (sICAM-1) concentrations in plasma were determined using quantitative sandwich enzyme immunoassay kits (R&D Systems, Abingdon, UK).

Biochemical measurements were conducted on 12-h fasted plasma and serum, collected at each visit and stored at −80 C until analysis in a single batch at the end of the study. Serum calcium adjusted for albumin, and creatinine concentrations were measured (ADIVA 1800 Chemistry System) for safety monitoring. Interassay coefficients of variation were less than 10% for the lipid, insulin, glucose, hsCRP, IL-6, and sICAM-1 assays.

Blood pressure

At each visit, blood pressure was measured with an OMRON705CP sphygmomanometer (Omron, Hertfordshire, UK) according to the guidelines from the British Hypertension Society (23). Three readings were taken from the left arm with participants in the supine position, and the mean of readings two and three was taken as the outcome. Overall, the three measurements varied by less than 5%.

Total 25(OH)D and PTH

$25(OH)D_2$, $25(OH)D_3$, and deuterated internal standard were extracted from serum samples. Potential interfering compounds were removed by initial elution with 50% methanol followed by elution of the vitamins using 10% tetrahydrofuran in acetonitrile. Dried extracts were reconstituted before injection into a high-pressure liquid chromatography tandem mass spectrometer in the multiple reaction mode. The following transitions (mass-to-charge ratio) were used: $413.2 > 395.3$, $401.1 > 383.3$, and $407.5 > 107.2$ for $25(OH)D_2$, $25(OH)D_3$, and hexadeuterated $25(OH)D_3$, respectively. Interassay coefficients of variation for the assay were less than 10% for both $25(OH)D_2$ and $25(OH)D_3$. Total 25(OH)D was calculated from combining $25(OH)D_2$ and $25(OH)D_3$.

Plasma samples were analyzed for PTH using an electrochemiluminescent immunoassay on a Modular Analytics E170 analyzer (Roche Diagnostics, Burgess Hill, UK). The inter-/intraassay coefficient of variation was less than 4% between 1 and 30 pmol/liter. The assay sensitivity (replicates of the zero standard) was 0.8 pmol/liter.

Potential confounding outcomes

At baseline and 12-month study visits, whole body scans were performed by dual-energy x-ray absorptiometry (Lunar iDXA; GE Medical Systems Ltd., Hertfordshire, UK), participants were weighed on balance scales (Tanita Europe BV, Amsterdam, The

Netherlands), height was measured using a stadiometer (Holtain Ltd., Crymych, UK), and waist circumference was measured using a metallic tape. Physical activity level and diet were assessed with validated questionnaires (24, 25) at every visit. UVB exposure (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org) was measured using UVB light-sensitive badges (University of Manchester, UK) pinned to the lapel of the study participants' outside coats for 1 wk after each study visit. The weekly standard erythemal dose (SED), a measure of the erythemal effectiveness of a UV exposure, was then calculated (26). At each visit, questions were asked on the extent of skin exposure to sunlight, including the body surface area exposed.

Statistical analyses

Sample size was based on primary outcomes of total and LDL cholesterol, APO A-1, and APO B_{100}. Interindividual variation in these measures has been shown to range from 10–20% (27, 28). Covariate adjustment would reduce this to 5–10%, indicating that 75 participants per treatment group would give sufficient experimental power (90%) to detect intervention effects of 5–7%. Three hundred participants would allow for a dropout rate of 25%. Using SPSS for Windows software (version 18.0; SPSS Inc., Chicago, IL) non-normally distributed data were natural logarithm-transformed before analysis. Differences in primary outcomes from baseline to follow-up at 12 months were examined between treatment groups in an intention-to-treat analysis (one-way ANOVA of differences from baseline). In a prespecified per-protocol analysis, differences in primary outcomes between treatment groups across study visits were examined by repeated measures ANOVA. Mixed modeling was employed where appropriate to explore the confounding effects of independent variables at each study visit, such as serum total 25(OH)D, plasma PTH, heart rate, body weight, physical activity level, grip strength, weekly SED, sunlight exposure assessed by questionnaire, dietary potassium intake, serum calcium (adjusted for albumin) concentrations, and baseline adipose tissue distribution. Those variables missing completely at random are accounted for by the initial analysis determining no differences between the participants with complete data and those with missing data. The analysis that was performed (mixed linear modeling) also assumes that any missing data are missing at random. Compliance was estimated at every study visit by capsule count.

Results

Figure 1 shows the flow of participants in the trial. A total of 305 women were randomly assigned to one of three study interventions. In total, 40 withdrew (six due to personal reasons, 34 due to clinical reasons). Overall compliance was 95% (data not shown).

Baseline data

Baseline characteristics (Table 1) were similar between treatment groups, with the exception of mean serum concentrations of total and LDL cholesterol and APO B_{100}, which were significantly ($P < 0.05$) lower in participants receiving placebo.

Effects of treatment

Our intention-to-treat analysis shows comparative changes with 95% CI in primary study outcomes for each treatment group between baseline and 12 months (Table 2). At 12 months, there was no difference in mean change for any of our primary outcomes of CVD risk factors between treatment groups (one-way ANOVA).

Supplementation with vitamin D_3 increased serum total 25(OH)D rapidly in the first 2 months to more than 50 nmol/liter for both the 400- and 1000-IU groups (Table 3). The percentage of participants with serum total 25(OH)D concentration greater than 50 nmol/liter at 12 months increased from 15% at baseline to 80% for the 400 IU vitamin D_3 group and to 93% for the 1000 IU vitamin D_3 group (compared with <15% for the placebo group). At 12 months, the incremental increase in total 25(OH)D in the 1000-IU group over the 400-IU group was small, indicating a nonlinear dose response to supplementation. Significant differences in mean and median concentrations of total 25(OH)D and PTH, respectively, were observed between study visits ($P < 0.001$) and treatment groups ($P < 0.001$) (repeated measures ANOVA; Table 3). Peak plasma PTH reductions were significantly greater from vitamin D_3 supplementation compared with placebo ($P < 0.005$; one-way ANOVA; data not shown). Median PTH concentrations remained suppressed in both the 400- and 1000-IU groups throughout the study, and a reduction in total 25(OH)D during winter induced an increase in median PTH concentration for participants receiving placebo (Table 3).

Our per-protocol analysis showed significant seasonal variation in mean lipid (total, HDL, and LDL cholesterol, triglycerides, APO A-1, and APO B_{100}), glucose, and median inflammatory marker (hsCRP, IL-6, and sICAM-1) concentrations (repeated measures ANOVA; $P < 0.001$; Table 3). After adjustment for potential confounders, differences between study visits for these outcomes were not significant. There was no difference between treatment groups (repeated measures ANOVA) for total, HDL, and LDL cholesterol, triglyceride, and APO A-1 concentrations (Table 3). Median concentrations of hsCRP, IL-6, and sICAM-1 were unaffected by study treatment (Table 3). There were no treatment effects on glucose and insulin mean concentrations or estimates of insulin resistance (HOMA-IR), (Table 3). Significant differences in serum APO B_{100} concentrations were observed (repeated measures ANOVA; $P = 0.04$) between treatment groups (Table 3), and these effects remained ($P = 0.03$) after adjustment for potential confounders (Table 3).

TABLE 1. Participant characteristics at baseline

Characteristics	400 IU vitamin D_3	1000 IU vitamin D_3	Placebo	*P* value[a]
n	102	101	102	
Age (yr)	63.5 (1.9)	64.1 (2.3)	63.9 (2.3)	0.17
Weight (kg)	68.6 (12.7)	69.6 (11.9)	69.3 (12.5)	0.84
BMI (kg/m^2)	26.6 (4.2)	26.8 (4.2)	26.6 (4.4)	0.96
Waist circumference (cm)	85.5 (10.1)	87.2 (11.4)	86.0 (11.2)	0.63
Fat mass (kg)	27.9 (8.1)	27.9 (8.1)	27.7 (8.3)	0.74
Lean mass (kg)	37.4 (4.9)	38.3 (4.3)	38.3 (4.3)	0.87
Bone mass (kg)	2.18 (0.30)	2.21 (0.27)	2.22 (0.29)	0.48
Serum creatinine (μmol/liter)	63.6 (9.2)	66.1 (8.8)	65.1 (11.4)	0.20
Serum urea (mmol/liter)	5.58 (1.44)	5.59 (1.08)	5.43 (1.10)	0.61
Serum calcium (mmol/liter)	2.34 (0.07)	2.34 (0.08)	2.35 (0.07)	0.36
Serum 25(OH)D (nmol/liter)	32.74 (12.9)	32.41 (13.8)	36.18 (17.1)	0.13
Plasma PTH (pmol/liter), median (IQR)	1.52 (0.34)	1.55 (0.35)	1.65 (0.34)	0.10
Lipid values (mg/dl)				
Total cholesterol	246.7 (34.8)	248.7 (34.8)	237.5 (30.9)	0.02[c]
HDL cholesterol	74.9 (15.4)	76.8 (15.4)	75.3 (19.3)	0.75
LDL cholesterol	152.1 (30.9)	151.0 (30.9)	141.7 (27.0)	0.003[c]
Triglycerides	106.2 (44.3)	106.2 (44.3)	106.2 (53.1)	0.95
APO A-1	182.0 (20.0)	183.0 (20.0)	181.0 (30.0)	0.92
APO B_{100}	104.0 (20.0)	105.0 (20.0)	99.0 (20.0)	0.007[c]
Insulin resistance				
Glucose (mg/dl)	89.9 (9.0)	90.5 (9.0)	90.8 (7.7)	0.89
Insulin (mU/liter)	5.2 (5.1)	5.40 (5.4)	5.80 (6.4)	0.75
HOMA-IR	1.16 (0.12)	1.34 (0.19)	1.35 (0.19)	0.69
Inflammatory markers, median (IQR)				
hsCRP (mg/liter)	1.15 (2.10)	1.10 (3.10)	1.45 (3.33)	0.64
sICAM-1 (mg/ml)	0.26 (0.14)	0.24 (0.10)	0.25 (0.13)	0.38
IL-6 (pg/ml)	1.59 (1.63)	1.57 (1.53)	1.62 (1.62)	0.84
Blood pressure (mm Hg)				
Systolic	128.16 (13.8)	129.15 (15.6)	128.18 (13.3)	0.60
Diastolic	77.68 (7.3)	76.96 (8.1)	77.70 (7.8)	0.85
Heart rate (BPM)	67.1 (8.7)	65.2 (7.7)	64.4 (9.4)	0.08
UVB exposure, weekly	0.5 (0.7)	0.5 (1.0)	0.5 (0.8)	0.40
SED[b]	n = 95	n = 99	n = 99	
Socioeconomic status (%)[d]				
I	29.7	23.8	30.4	0.48
II	45.5	58.4	48.0	
III	4.0	5.0	4.9	
IV	11.9	6.9	13.7	
V–VI	9.0	5.9	3.0	

Data are presented as mean (SD), unless otherwise stated. To convert cholesterol values to mmol/liter, multiply by 0.0259. To convert triglyceride values to mmol/liter, multiply by 0.0113. To convert APO values to g/liter, multiply by 0.01. To convert glucose values to mmol/liter, multiply by 0.0555. BMI was calculated as weight in kilograms divided by height in meters squared. IQR, Interquartile range; BPM, beats per minute.

[a] Determined with one-way ANOVA for continuous variables and with χ^2 test for socioeconomic status.

[b] Missing values for UVB exposure at baseline due to study participants' not returning dosimeter badges after visit.

[c] Differences between treatment groups were not significant after adjustment for multiple testing.

[d] Based on postcode classification, where "I" represents the most affluent and "VI" represents the most deprived.

Significant differences in mean blood pressure measurements between study visits (repeated measures ANOVA; $P < 0.001$; Fig. 2) were observed, with mean reductions in systolic blood pressure from winter (January-March 2009) to summer (July-September 2009) study visits of −7.6, −5.8, and −5.8 mm Hg (Fig. 2A) for 400 and 1000 IU vitamin D_3 and placebo, respectively. A similar reduction (−3.1, −2.1, and −3.0 mm Hg, respectively) was observed for diastolic blood pressure (Fig. 2B). None of the treatments significantly affected systolic or diastolic blood pressure (see Fig. 2 legend). Mixed model analysis (Table 4) showed that study visit (season), heart rate, serum calcium concentration, body weight, and weekly SED were significant ($P < 0.05$) predictors of systolic and diastolic absolute blood pressure.

Adverse events

Fifty-two adverse events were reported (17, 15, and 20 in the 400 and 1000 IU vitamin D_3 and placebo groups, respectively; Supplemental Table 2). These included transient complaints such as dizziness, headache, nausea, or joint pain. Nineteen serious adverse

TABLE 2. Study visit data, vitamin D_3 effects on CVD risk: change between baseline and follow-up, intention-to-treat analysis[a]

	Change (95% CI) from baseline to 12 months	*P* value[b]
Serum total 25(OH)D (nmol/liter)		<0.001
400 IU vitamin D_3, n = 97	+33.04 (29.03 to 37.06)	
1000 IU vitamin D_3, n = 95	+42.90 (39.09 to 46.72)	
Placebo, n = 100	−2.72 (−5.15 to −0.29)	
Blood pressure (mm Hg)		
Systolic		0.87
400 IU vitamin D_3, n = 96	−2.2 (−3.3 to −0.7)	
1000 IU vitamin D_3, n = 95	−1.5 (−3.8 to 0.7)	
Placebo, n = 98	−2.4 (−4.5 to −0.2)	
Diastolic		0.11
400 IU vitamin D_3, n = 97	−2.5 (−3.6 to −1.4)	
1000 IU vitamin D_3, n = 96	−0.9 (−2.0 to 0.2)	
Placebo, n = 100	−2.1 (−3.1 to −1.0)	
Lipid values (mg/dl)		
Total cholesterol		0.49
400 IU vitamin D_3, n = 97	−5.8 (−10.0 to −1.5)	
1000 IU vitamin D_3, n = 95	−5.0 (−10.0 to −0.4)	
Placebo, n = 100	−2.3 (−6.2 to 1.5)	
HDL cholesterol		0.76
400 IU vitamin D_3, n = 97	−2.7 (−3.1 to −1.2)	
1000 IU vitamin D_3, n = 95	−2.3 (−4.3 to −0.8)	
Placebo, n = 100	−1.9 (−3.5 to 0)	
LDL cholesterol		0.56
400 IU vitamin D_3, n = 97	−3.5 (−7.0 to −0.4)	
1000 IU vitamin D_3, n = 93	−3.1 (−7.0 to −0.8)	
Placebo, n = 100	−1.2 (−4.6 to 2.3)	
Triglycerides		0.89
400 IU vitamin D_3, n = 97	−0.3 (−7.1 to 6.2)	
1000 IU vitamin D_3, n = 95	+1.8 (−5.3 to 8.0)	
Placebo, n = 100	+1.8 (−3.5 to 7.1)	
APO A-1		0.59
400 IU vitamin D_3, n = 97	−5.0 (−8.0 to −2.0)	
1000 IU vitamin D_3, n = 95	−3.0 (−7.0 to 0)	
Placebo, n = 100	−3.0 (−6.0 to 0.1)	
APO B_{100}		0.44
400 IU vitamin D_3, n = 97	−2.0 (−4.0 to 0.2)	
1000 IU vitamin D_3, n = 95	−1.0 (−4.0 to 1.0)	
Placebo, n = 100	0 (−2.0 to 2.0)	
HOMA-IR		0.89
400 IU vitamin D_3, n = 97	0 (−0.16 to 0.16)	
1000 IU vitamin D_3, n = 94	−0.05 (−0.27 to 0.16)	
Placebo, n = 99	0 (−0.18 to 0.17)	
Inflammatory markers		
hsCRP (mg/liter)		0.73
400 IU vitamin D_3, n = 97	+0.94 (−1.19 to 3.06)	
1000 IU vitamin D_3, n = 95	+1.25 (−0.79 to 3.29)	
Placebo, n = 100	+0.24 (−0.93 to 1.41)	
sICAM-1 (mg/ml)		0.67
400 IU vitamin D_3, n = 96	−0.001 (−0.02 to 0.02)	
1000 IU vitamin D_3, n = 97	+0.01 (−0.01 to 0.03)	
Placebo, n = 99	+0.003 (−0.02 to 0.03)	
IL-6 (pg/ml)		0.84
400 IU vitamin D_3, n = 95	+0.13 (−0.28 to 0.53)	
1000 IU vitamin D_3, n = 94	+0.23 (−0.19 to 0.65)	
Placebo, n = 96	+0.31 (−0.14 to 0.75)	

Data are presented as mean change (95% CI). To convert cholesterol values to mmol/liter, multiply by 0.0259. To convert triglyceride values to mmol/liter, multiply by 0.0113. To convert APO values to g/liter, multiply by 0.01. There was no difference in percentage change over 12 months for any of our primary outcomes of CVD risk factors between treatment groups (one-way ANOVA), nor were there any differences for any of our primary outcomes at 12 months between treatment groups (analysis of covariance) adjusting for baseline values.

[a] Includes all participants who changed medications or discontinued the intervention.

[b] Between treatments determined by one-way ANOVA.

TABLE 3. Study visit data, vitamin D_3 effects on CVD risk: time and treatment effects

	Baseline	2 months	4 months	6 months	8 months	10 months	12 months	Unadjusted *P* (time[a]; treatment[b])	Adjusted *P* (time[a]; treatment[b])
Serum total 25(OH)D (nmol/liter)								<0.001; <0.001	<0.001; <0.001
400 IU, n = 83	33.27 (13.2)	57.33 (14.1)	64.60 (15.3)	68.37 (15.6)	69.44 (17.0)	65.85 (16.5)	64.86 (19.8)		
1000 IU, n = 87	33.35 (13.9)	63.46 (16.2)	70.53 (16.8)	77.20 (18.4)	77.75 (20.3)	75.76 (18.9)	75.66 (19.1)		
Placebo, n = 87	36.30 (16.4)	34.88 (14.6)	44.08 (18.4)	55.82 (18.4)	48.95 (20.2)	38.73 (17.5)	32.43 (14.7)		
Plasma PTH (pmol/liter), median (IQR)								<0.001; <0.001	<0.001; <0.001
400 IU, n = 75	1.53 (0.34)	1.43 (0.37)	1.39 (0.36)	1.42 (0.32)	1.41 (0.43)	1.48 (0.34)	1.44 (0.42)		
1000 IU, n = 70	1.55 (0.35)	1.50 (0.38)	1.44 (0.30)	1.46 (0.21)	1.47 (0.32)	1.46 (0.36)	1.44 (0.30)		
Placebo, n = 77	1.65 (0.34)	1.61 (0.34)	1.59 (0.31)	1.58 (0.24)	1.57 (0.33)	1.61 (0.39)	1.59 (0.33)		
Lipid values (mg/dl)									
Total cholesterol								<0.001; 0.14	0.38; 0.09
400 IU, n = 82	246.7 (34.8)	236.7 (34.8)	239.4 (38.6)	235.1 (34.8)	239.4 (34.8)	236.7 (34.8)	242.5 (34.8)		
1000 IU, n = 84	248.7 (34.8)	241.3 (34.8)	241.7 (34.8)	240.9 (34.8)	246.3 (34.8)	245.6 (38.6)	243.6 (34.8)		
Placebo, n = 82	237.5 (30.9)	231.7 (30.9)	229.0 (30.9)	231.7 (27.0)	231.7 (30.9)	229.7 (27.0)	234.8 (30.9)		
HDL cholesterol								<0.001; 0.97	0.80; 0.94
400 IU, n = 84	74.9 (15.4)	72.2 (15.4)	72.2 (15.4)	71.4 (15.4)	72.2 (15.4)	71.4 (15.4)	72.6 (15.4)		
1000 IU, n = 82	76.8 (15.4)	74.5 (15.4)	74.9 (15.4)	73.3 (15.4)	74.5 (15.4)	74.9 (15.4)	74.1 (15.4)		
Placebo, n = 84	75.3 (19.3)	73.4 (19.3)	74.1 (19.3)	73.0 (19.3)	73.0 (15.4)	73.8 (19.3)	74.1 (19.3)		
LDL cholesterol								<0.001; 0.28	0.53; 0.21
400 IU, n = 79	152.1 (30.9)	144.8 (30.9)	147.9 (34.8)	145.2 (30.9)	147.9 (30.9)	145.6 (23.2)	149.4 (30.9)		
1000 IU, n = 82	160.0 (34.8)	146.3 (34.8)	147.5 (34.8)	147.1 (30.9)	150.6 (30.9)	149.4 (34.8)	147.9 (34.8)		
Placebo, n = 81	141.7 (27.0)	138.2 (27.0)	136.3 (27.0)	137.8 (23.2)	138.6 (27.0)	136.3 (27.0)	139.8 (27.0)		
Triglycerides								<0.001; 0.22	0.16; 0.39
400 IU, n = 82	102.7 (35.4)	99.1 (44.3)	98.2 (44.3)	95.6 (44.3)	99.1 (35.4)	100.9 (44.3)	105.3 (44.3)		
1000 IU, n = 84	106.2 (44.3)	102.7 (44.3)	100.9 (44.3)	102.7 (53.1)	106.2 (44.3)	106.2 (53.1)	108.9 (44.3)		
Placebo, n = 82	106.2 (53.1)	107.1 (62.0)	97.4 (53.1)	108.9 (62.0)	107.1 (70.8)	104.4 (62.0)	108.9 (62.0)		
APO A-1								<0.001; 0.74	0.46; 0.46
400 IU, n = 82	182.0 (20.0)	176.0 (20.0)	176.0 (20.0)	174.0 (20.0)	176.0 (20.0)	175.0 (20.0)	178.0 (20.0)		
1000 IU, n = 84	183.0 (20.0)	181.0 (30.0)	180.0 (30.0)	178.0 (20.0)	180.0 (30.0)	180.0 (20.0)	179.0 (20.0)		
Placebo, n = 82	181.0 (30.0)	178.0 (30.0)	179.0 (30.0)	177.0 (30.0)	177.0 (30.0)	178.0 (20.0)	179.0 (20.0)		
APO B_{100}								<0.001; 0.04	0.80; 0.03
400 IU, n = 82	104.0 (20.0)	100.0 (20.0)	102.0 (2.0)	100.0 (20.0)	102.0 (20.0)	100.0 (20.0)	103.0 (20.0)		
1000 IU, n = 84	105.0 (20.0)	102.0 (20.0)	104.0 (20.0)	103.0 (20.0)	105.0 (20.0)	105.0 (20.0)	104.0 (20.0)		
Placebo, n = 82	99.0 (20.0)	97.0 (20.0)	96.0 (20.0)	98.0 (20.0)	98.0 (20.0)	96.0 (20.0)	99.0 (20.0)		
Glucose (mg/dl)								<0.001; 0.38	0.31; 0.23
400 IU, n = 81	87.4 (7.2)	90.3 (7.2)	82.2 (9.0)	93.7 (7.2)	84.1 (7.2)	89.9 (7.2)	88.3 (9.0)		
1000 IU, n = 83	90.5 (9.0)	91.5 (9.0)	90.6 (9.0)	89.6 (9.0)	89.9 (9.0)	90.8 (9.0)	89.4 (9.0)		
Placebo, n = 78	90.8 (7.2)	90.3 (9.0)	90.5 (9.0)	90.1 (7.2)	88.8 (9.0)	89.6 (9.0)	88.5 (9.0)		
Insulin (mU/liter)								0.18; 0.32	0.92; 0.13
400 IU, n = 82	4.85 (4.5)	5.01 (4.9)	4.56 (4.4)	5.20 (5.0)	4.67 (4.9)	4.99 (4.2)	4.90 (4.6)		
1000 IU, n = 84	5.01 (5.5)	5.24 (8.2)	5.00 (7.8)	4.86 (5.9)	5.47 (9.3)	5.45 (9.8)	5.07 (9.5)		
Placebo, n = 81	5.40 (5.7)	5.35 (6.4)	5.16 (7.2)	5.50 (7.0)	5.26 (5.9)	5.31 (7.1)	5.04 (6.4)		
HOMA-IR								0.15; 0.39	0.74; 0.14
400 IU, n = 81	1.16 (1.1)	1.19 (1.2)	1.08 (1.1)	1.23 (1.2)	1.10 (1.2)	1.19 (1.1)	1.17 (1.2)		
1000 IU, n = 81	1.34 (1.7)	1.28 (2.0)	1.23 (1.8)	1.16 (1.4)	1.33 (2.2)	1.32 (2.3)	1.18 (2.0)		
Placebo, n = 74	1.35 (1.6)	1.36 (1.9)	1.32 (2.3)	1.39 (2.0)	1.29 (1.5)	1.34 (2.0)	1.25 (1.7)		
Inflammatory markers, median (IQR)									
hsCRP (mg/liter)								<0.001; 0.29	0.09; 0.26
400 IU, n = 80	1.15 (2.10)	1.15 (2.10)	1.05 (2.23)	1.15 (2.28)	0.90 (2.50)	0.85 (1.83)	1.20 (2.75)		
1000 IU, n = 80	1.10 (3.10)	1.20 (2.90)	1.00 (2.50)	1.00 (3.00)	1.30 (3.20)	1.60 (3.90)	1.40 (4.50)		
Placebo, n = 79	1.45 (3.33)	0.90 (2.95)	0.95 (2.10)	1.20 (2.68)	1.05 (2.60)	0.95 (3.60)	1.10 (2.95)		
sICAM-1 (μg/ml)								0.002; 0.25	0.36; 0.11
400 IU, n = 74	0.26 (0.14)	0.25 (0.14)	0.25 (0.11)	0.23 (0.14)	0.23 (0.11)	0.26 (0.11)	0.26 (0.11)		
1000 IU, n = 76	0.24 (0.10)	0.23 (0.10)	0.22 (0.10)	0.26 (0.15)	0.24 (0.14)	0.25 (0.13)	0.25 (0.13)		
Placebo, n = 79	0.25 (0.13)	0.23 (0.11)	0.25 (0.13)	0.25 (0.09)	0.25 (0.11)	0.25 (0.13)	0.26 (0.12)		
IL-6 (pg/ml)								<0.001; 0.10	0.78; 0.08
400 IU, n = 75	1.59 (1.63)	1.65 (1.54)	1.47 (1.24)	1.56 (1.11)	1.43 (1.17)	1.29 (1.15)	1.58 (1.17)		
1000 IU, n = 76	1.57 (1.53)	1.56 (1.43)	1.49 (1.14)	1.49 (1.17)	1.67 (1.34)	1.86 (1.99)	1.69 (2.00)		
Placebo, n = 79	1.62 (1.90)	1.35 (1.61)	1.61 (1.39)	1.53 (1.60)	1.51 (1.62)	1.79 (1.62)	1.76 (2.07)		

Data are presented as mean (SD), unless otherwise stated. To convert cholesterol values to mmol/liter, multiply by 0.0259. To convert triglyceride values to mmol/liter, multiply by 0.0113. To convert APO values to g/liter, multiply by 0.01. To convert glucose values to mmol/liter, multiply by 0.0555. IQR, interquartile range. Results of repeated measures ANOVA following adjustment for potential confounders; baseline measurements of weight, heart rate, serum calcium (adjusted for albumin), serum total 25(OH)D, physical activity level, waist circumference, grip strength. Additional adjustments for PTH and estimated dietary intakes of vitamin D and calcium did not change the outcome. Two participants who were taking dietary supplements containing calcium were excluded in a sensitivity analysis. This did not change the outcome.

[a] Between visits, determined with repeated measures ANOVA.

[b] Between treatments, determined with repeated measures ANOVA.

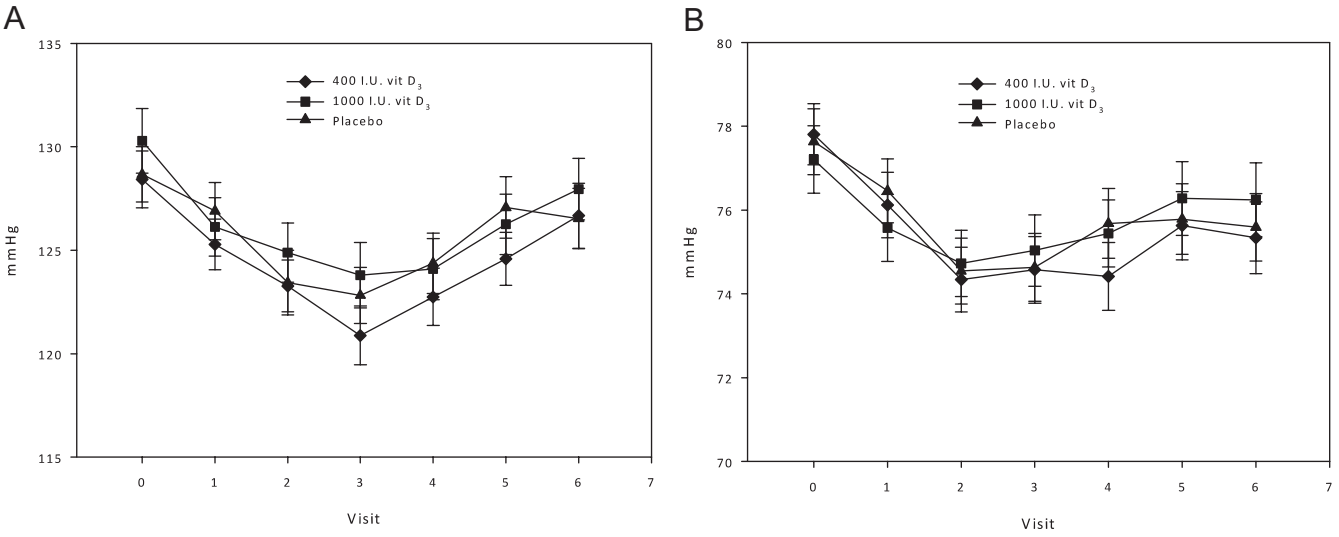

FIG. 2. Mean (SEM) systolic blood pressure (A) and diastolic blood pressure (B) according to treatment group. There was no difference between treatment groups for mean measures of systolic ($P = 0.84$) and diastolic ($P = 0.85$) blood pressure (repeated-measures ANOVA). Significant differences in mean systolic and diastolic blood pressure measurements were observed between study visits (repeated measures ANOVA; $P < 0.001$) (Table 5).

events (life threatening or requiring inpatient hospitalization) were reported (7, 8, and 4 in the 400 and 1000 IU vitamin D_3 and placebo groups, respectively; Supplemental Table 2). None were deemed to be related to study interventions by the Data Monitoring Committee.

Discussion

Establishing an effect contribution of insufficient vitamin D exposure on risk factors for CVD would impact on clinical practice. However, fervor for promising interventions can often overtake available evidence. The limited available data from RCT with intervention formulations containing vitamin D_2, D_3, or vitamin D analogs, focusing on physiological markers of CVD risk in human subjects, are contradictory and of varying quality (1, 2, 16–20). Uncertainties relating to vitamin D requirements further highlight a critical requirement for research in this area.

Systemic markers of CVD risk

Daily supplementation with vitamin D_3 (400 or 1000 IU) over 1 yr had no effect on total, HDL, and LDL cholesterol, triglycerides, APO A-1, estimates of insulin resistance, or inflammatory markers. Results were consistent when analyzed using an intention-to-treat or prespecified per-protocol analysis. Although the positive effect of vitamin D_3 supplementation on serum APO B_{100} concentrations may be consistent with a cardiovascular benefit, the reduction was small, and we would question its clinical significance. The lack of effect of vitamin D_3 on systemic markers of CVD risk contrasts with data from two key studies (29, 30). Serum concentrations of triglycerides ($P < 0.001$) and the proinflammatory cytokine TNF-α ($P = 0.049$) were reduced after supplementation for 1 yr with vitamin D_3 (3320 IU/d) compared with placebo (n = 100 per group) (29). Positive effects on cytokine profile have also been observed in patients with congestive heart failure (30) (n = 123) who received either 2000 IU vitamin D_3 plus 500 mg of calcium per day or placebo plus 500 mg of calcium for 9 months.

It is possible that our null finding was a result of our relatively healthy study cohort who were free of most diseases and not on vascular medications. Mean serum HDL cholesterol concentrations in our study population were consistently relatively high, in comparison to mean values for the UK population (63 mg/dl for women aged 19–64; National Diet and Nutrition Survey supplementary report on blood analytes: combined data for years 2008–2009 and 2009–2010) (31). Additionally, vitamin D_3 doses of 400 or 1000 IU may have been too low to have beneficial effects on surrogate markers of CVD risk. However, our participants could be considered at risk of vitamin D deficiency, having particularly low serum total 25(OH)D concentrations at baseline when compared with current recommendations (32, 33).

Seasonal variation in blood pressure

We found significant seasonal effects on blood pressure measurements, observed in all treatment groups with clinically relevant reductions in systolic blood pressure from winter to summer (34). This seasonal effect was independent of other significant predictors of blood pressure and other potential confounding variables. Seasonal variation

TABLE 4. Results of mixed model analysis to identify predictors of blood pressure for all participants (n = 265)

Independent variable[a]	Estimate[b]	SE	95% CI	*P* value
Systolic blood pressure (mm Hg)				
Intercept	445.396	87.625	273.512 to 617.280	<0.001
Baseline visit (Jan-Mar 09)[c]	0	0		
Visit 1 (Mar–May 09)	0.758	4.845	−8.779 to 10.295	0.88
Visit 2 (May–Jul 09)	−1.759	5.371	−12.327 to 8.809	0.74
Visit 3 (Jul–Sep 09)	−11.024	5.115	−21.087 to −0.960	0.03
Visit 4 (Sep–Nov 09)	−14.679	4.372	−23.277 to −6.080	0.001
Visit 5 (Nov 09–Jan 10)	−7.182	5.389	−17.778 to 3.413	0.18
Visit 6 (Jan–Mar 10)	1.578	5.282	−8.812 to 11.967	0.77
Weight (kg)	−2.025	0.852	−3.697 to −0.354	0.02
Serum calcium (mmol/liter)	−136.575	37.375	−209.890 to −63.260	<0.001
Heart rate (BPM)	−2.493	0.983	−4.421 to −0.565	0.01
Sunlight exposure, weekly SED	−0.090	0.042	−0.173 to −0.007	0.03
Baseline visit∗heart rate (BPM)[c]	0	0		
Visit 1∗heart rate (BPM)	−0.049	0.073	−0.193 to 0.095	0.50
Visit 2∗heart rate (BPM)	−0.044	0.081	−0.205 to 0.116	0.59
Visit 3∗heart rate (BPM)	0.083	0.078	−0.070 to 0.236	0.29
Visit 4∗heart rate (BPM)	0.150	0.066	0.020 to 0.280	0.02
Visit 5∗heart rate (BPM)	0.069	0.081	−0.090 to 0.228	0.39
Visit 6∗heart rate (BPM)	−0.054	0.080	−0.211 to 0.103	0.50
Weight (kg)∗serum calcium (mmol/liter)	0.967	0.364	0.252 to 1.682	0.01
Serum calcium (mmol/liter)∗heart rate (BPM)	0.980	0.419	0.158 to 1.802	0.02
Diastolic blood pressure (mm Hg)				
Intercept	139.207	32.571	75.317 to 203.096	<0.001
Baseline visit[c]	0	0		
Visit 1	1.987	2.711	−3.348 to 7.322	0.46
Visit 2	−0.298	2.866	−5.937 to 5.340	0.92
Visit 3	−2.876	2.672	−8.133 to 2.381	0.28
Visit 4	5.613	2.172	1.340 to 9.886	0.01
Visit 5	−0.614	2.654	−5.834 to 4.605	0.82
Visit 6	4.066	2.687	−1.220 to 9.352	0.13
Weight (kg)	−1.027	0.465		0.03
Serum calcium (mol/liter)	−26.831	13.685		0.05
Heart rate (BPM)	−0.159	0.106		0.13
Sunlight exposure, weekly SED	−0.064	0.023		0.01
Baseline visit∗heart rate (BPM)[c]	0	0		
Visit 1∗heart rate (BPM)	−0.049	0.041		0.23
Visit 2∗heart rate (BPM)	−0.031	0.043		0.48
Visit 3∗heart rate (BPM)	0.012	0.041		0.77
Visit 4∗heart rate (BPM)	−0.119	0.033		<0.001
Visit 5∗heart rate (BPM)	−0.014	0.040		0.72
Visit 6∗heart rate (BPM)	−0.089	0.041		0.03
Weight (kg)∗serum calcium (mmol/liter)	0.396	0.196		0.04
Weight (kg)∗heart rate (BPM)	0.004	0.001		0.01

BPM, Beats per minute.

[a] Serum total 25(OH)D, plasma PTH, dietary potassium intake, physical activity level, extent of skin exposure to sunlight, baseline trunk fat mass, and baseline appendicular fat mass were added separately to the model in a repeated measures analysis. These factors were not significant predictors of either systolic or diastolic blood pressure. We conducted a sensitivity analysis for PTH to include a factor excluding participants with the highest and lowest 2.5% of plasma PTH concentrations. This factor was not a significant predictor of either systolic or diastolic blood pressure. Additional analyses to include estimated dietary intakes of vitamin D and calcium did not change the outcome. These factors were not significant predictors of either systolic or diastolic blood pressure.

[b] From our model, by inserting mean (observed) values for predictor variables (weight = 68.3 kg; serum calcium adjusted for albumin = 2.34 mmol/liter; heart rate = 65 BPM, weekly SED = 0.9), an estimate of systolic blood pressure at baseline would be: 445.4 − (2.025∗68.3) − (136.575∗2.34) − (2.493∗65) − (0.09∗0.9) + 0.967 (68.3∗2.34) + 0.98 (2.34∗65) = 129.0 mm Hg.

[c] These parameters were set to zero in the model.

in blood pressure has been described in several populations (35–37). Our findings support observational data for a large cohort of older UK women (aged 55–64 yr) showing an inverse association between outside air temperature and blood pressure measurements (mean deviation in systolic blood pressure associated with a 20 C difference in maximum daily temperatures was 6.3 mm Hg) (35). To our knowledge, this is the first vitamin D intervention study to highlight a seasonal variation in blood pressure that is independent of vitamin D dose; in studies

TABLE 5. Blood pressure [mean (SD)] at each visit in all participants (n = 265)

Blood pressure, mm Hg	Jan–Mar 2009	Mar–May 2009	May–Jul 2009	Jul–Sep 2009	Sep–Nov 2009	Nov 09–Jan 2010	Jan–Mar 2010
Systolic	129.1 (14.2)	126.1 (13.2)	123.9 (13.8)	122.5 (14.0)	123.7 (13.7)	126.0 (13.3)	127.1 (14.1)
Diastolic	77.6 (7.7)	76.1 (7.7)	74.5 (7.6)	74.7 (7.8)	75.2 (7.7)	75.9 (7.9)	75.7 (8.0)

of a shorter duration or where study visits are staggered, blood pressure effects were not observed (2, 19, 38). A number of trials (2, 16–18, 38) have attributed reductions in blood pressure to vitamin D, the majority of which have had small numbers and cannot be directly compared because they did not supplement with vitamin D_3. A meta-analysis of vitamin D supplementation effects on blood pressure (39) including pooled data from four RCT of vitamin D_3 found a 2.44 mm Hg (95% CI, −4.86 to −0.02) reduction for systolic blood pressure and no reduction for diastolic blood pressure, although only one reported a difference in systolic blood pressure change between treatment and placebo groups and all were of relatively short duration (5–15 wk).

Our study suggests that data attributing reductions in blood pressure to vitamin D or relating absolute blood pressure to vitamin D status assessed by total 25(OH)D are confounded by sunlight or seasonality, and consequently, other factors that are affected by sunlight or season and not vitamin D *per se* may be responsible for blood pressure changes. It is possible that serum total 25(OH)D between 36 and 56 nmol/liter is important in regulating blood pressure because this was the change in total 25(OH)D concentration observed in the placebo group from winter to summer, whereas vitamin D_3 treatment [which resulted in higher serum total 25(OH)D concentrations] had no additional effect on blood pressure.

Strengths and limitations

Our RCT study design had a number of strengths. Visits were fixed at 2-month intervals over 1 yr, with all participants starting the intervention at the beginning of the year to capture any potential seasonal effects. Subject retention was excellent, as was study compliance. Participants had low total 25(OH)D concentrations at baseline, typical of older women of the region (40, 41).

We acknowledge some limitations. Our study was relatively small with only 305 participants, approximately 100 per treatment group, and duration of only 1 yr. We were not powered to examine incident cardiovascular events such as heart attack or stroke. Although we measured a comprehensive range of surrogate CVD risk factors, our study did not involve computerized tomography or ultrasound scanning. An effect of vitamin D_3 on carotid calcification or smooth muscle cell proliferation can therefore not be ruled out. Two doses of vitamin D_3 were given, 400 and 1000 IU/d. There is likely an optimal level of intake to meet vitamin D requirements. Based on bone health, the Institute of Medicine report (33) recommends 600 IU vitamin D_3 daily aimed at producing a population target of circulating total 25(OH)D of 50 nmol/liter, but the report concluded that there was insufficient evidence to make recommendations for non-bone health outcomes. It is possible that 1000 IU vitamin D_3 daily was not high enough for positive effects and that seasonal changes masked an effect of oral vitamin D. However, the nonlinear total 25(OH)D dose response we observed suggests that there is some resistance to 25(OH)D increase with increasing oral vitamin D. It is not known whether the additional vitamin D_3 in the higher dose group was not converted to 25(OH)D because it was stored, or degraded, or whether it was not fully absorbed. Further work is required to determine the fate of the additional vitamin D_3 in this group, which may be important when considering the risk benefit profile of "high dose" supplemental vitamin D. Although we found no effect of vitamin D_3 on markers of CVD risk, our results do not exclude potential benefits from supplementation in different patient populations such as a high-risk study population with multiple clinical risk factors, or in a large-scale study population assessing incident CVD events as primary prespecified outcomes. These possibilities require evaluation in prospective clinical trials.

In conclusion, in postmenopausal women living at 57° N, we found no evidence that daily supplementation with either 400 or 1000 IU vitamin D_3 over 1 yr altered conventional CVD risk factors. Season affected blood pressure independently of vitamin D dose. The confounding of seasonality should be recognized and addressed in future studies of vitamin D.

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