Vitamin D Therapy in Individuals With Prehypertension or Hypertension
The DAYLIGHT Trial

Pankaj Arora, MD; Yanna Song, MS; Jeffery Dusek, PhD; Gregory Plotnikoff, MD; Marc S. Sabatine, MD; Susan Cheng, MD; Andre Valcour, PhD; Heather Swales, MD; Beth Taylor, PhD; Erin Carney, BS; Derek Guanaga, BS; Joseph R. Young, BA; Courtney Karol, BS; Michael Torre, BS; Atum Azzahir, BA; Semerit M. Strachan, MD; Dillon C. O'Neill, BA; Myles Wolf, MD, MMSc; Frank Harrell, PhD; Christopher Newton-Cheh, MD, MPH; Thomas J. Wang, MD

Background—A large body of epidemiological and experimental evidence suggests that vitamin D deficiency may promote hypertension. This raises the possibility that vitamin D supplementation could be a simple intervention to reduce blood pressure, but data from prospective, randomized trials are limited.

Methods and Results—A double-blind, randomized, controlled trial was conducted at 4 sites in the United States. We enrolled 534 individuals 18 to 50 years of age with low vitamin D status (25-hydroxyvitamin D levels ≤25 ng/mL) and systolic blood pressure of 120 to 159 mm Hg. Participants were randomized to high-dose (4000 IU/d) versus low-dose (400 IU/d) oral vitamin D_3 for 6 months. The primary end point was change in mean 24-hour systolic blood pressure. Secondary end points included change in ambulatory diastolic blood pressure and clinic systolic and diastolic blood pressures. The median age was 38 years, and 62% of participants were men. Forty-six percent of participants were white, and 48% were black. The median 25-hydroxyvitamin D level at baseline was 15.3 ng/mL. Four-hundred fifty-five participants (85%) had at least 1 follow-up blood pressure measurement; 383 participants (72%) completed the full 6-month study. At the end of the study, there was no significant difference in the primary end point (change in mean 24-hour systolic blood pressure, −0.8 versus −1.6 mm Hg in the high-dose and low-dose arms; P=0.71) or in any of the secondary end points. Furthermore, there was no evidence of association between change in 25-hydroxyvitamin D and change in 24-hour systolic blood pressure at 6 months (Spearman correlation coefficient, −0.05, P=0.34). Results were consistent across prespecified subgroups.

Conclusions—Vitamin D supplementation did not reduce blood pressure in individuals with prehypertension or stage I hypertension and vitamin D deficiency. Our findings suggest that the association between vitamin D status and elevated blood pressure noted in observational studies is not causal.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01240512.

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Vitamin D deficiency is a common problem with implications for human health.[1] A large body of epidemiological evidence links vitamin D deficiency with a higher risk of cardiovascular disorders, including hypertension.[2,3] A meta-analysis of observational studies found that every 16-ng/mL decrease in vitamin D was associated with a 16% higher risk of hypertension.[4] A meta-analysis of population genetic studies suggested that polymorphisms related to

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From the Cardiology Division, Department of Medicine, University of Alabama at Birmingham, Birmingham (P.A.); Department of Biostatistics (Y.S., F.H.) and Division of Cardiovascular Medicine, Department of Medicine (D.C.O., T.J.W.), Vanderbilt University, Nashville, TN; Integrative Health Research Center, Abbott Northwestern Hospital, Minneapolis, MN (J.D., G.P.); Division of Cardiology, Department of Medicine, Brigham & Women's Hospital, Harvard Medical School, Boston, MA (M.S.S., S.C.); Esoterix Clinical Lab Services, LabCorp, Research Triangle Park, NC (A.V.); Division of Cardiology, Department of Medicine, Hartford Hospital, Hartford, CT (H.S., B.T.); Division of Cardiology, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston (E.C., D.G., J.R.Y., C.K., M.T., C.N.-C.); Division of Cardiology, Cultural Wellness Center, Minneapolis, MN (A.A., S.M.S.); and Division of Nephrology, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL (M.W.).

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Correspondence to Thomas J. Wang, MD, Division of Cardiovascular Medicine, Vanderbilt University Medical Center, 2220 Pierce Ave, 383 PRB, Nashville, TN 37232. E-mail thomas.j.wang@vanderbilt.edu

lower vitamin D status were associated with higher blood pressure.[5] Additionally, low vitamin D concentrations have been shown to predict future hypertension among individuals with normal blood pressure at baseline.[2] Experimental work provides further evidence of a link between vitamin D status and blood pressure. Vitamin D receptors are expressed throughout the cardiovascular system on vascular smooth muscle, endothelium, and cardiomyocytes.[6,7] Disruption of these receptors in animals is associated with elevated blood pressure, which can be normalized with vitamin D administration.[8]

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These observations raise the possibility that vitamin D supplementation could reduce blood pressure in humans. However, results of randomized, intervention trials have been conflicting, with some studies, but not others, suggesting a benefit.[9–14] In most of the trials, blood pressure was not the primary end point, nor was it measured with standardized protocols. These trials also typically randomized <150 participants and included a large proportion of individuals who were already on antihypertensive therapy. Importantly, very few nonwhite individuals have been included in prior studies, despite the high prevalence of both vitamin D deficiency and elevated blood pressure among minorities.

The absence of definitive data has led to calls for adequately powered, prospective, randomized trials of vitamin D supplementation and blood pressure.[15] Accordingly, we conducted DAYLIGHT (The Vitamin D Therapy in Individuals at High Risk of Hypertension Trial), a multicenter, randomized trial of vitamin D supplementation in a racially diverse sample of individuals with low vitamin D stores and elevated blood pressure.

Methods

Study Design

DAYLIGHT was a double-blind, multicenter, 6-month randomized trial of high-dose (4000 IU/d) versus low-dose (400 IU/d) vitamin D supplementation in individuals with prehypertension and untreated stage 1 hypertension and vitamin D deficiency. Participants were recruited at 4 sites (Massachusetts General Hospital [Boston, MA], Hartford Hospital [Hartford, CT], Cultural Wellness Center [Minneapolis, MN], and Abbott Northwestern Hospital [Minneapolis, MN]). Enrollment began in December 2010, and the final follow-up visit was performed in September 2013. The protocol was approved by the Institutional Review boards of Partners Healthcare, Hartford Hospital, and Allina Healthcare. Written informed consent was obtained from all participants.

Inclusion and Exclusion Criteria

The study enrolled participants between 18 and 50 years of age who had an averaged mean systolic blood pressure between 120 and 159 mm Hg and a diastolic blood pressure <99 mm Hg at 2 clinic visits. The other main inclusion criterion was a 25-hydroxyvitamin D level of ≤25 ng/mL at the screening visit.

Individuals were excluded if they had used any antihypertensive medication in the past 3 months, had used vitamin D supplementation in the past 3 months (defined as vitamin D found in a multivitamin or supplement totaling >400 IU/d), or had known cardiovascular disease (defined as prior myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass, or stroke). Other exclusion criteria are detailed in the online-only Data Supplement.

Vitamin D Supplementation

Vitamin D was administered through once-daily oral doses of vitamin D_3 (cholecalciferol), with a total of 4000 and 400 IU in the high-dose and low-dose arms, respectively. Administration was via gravity-metered dropper bottles to deliver a consistent dosage (Ddrops Co, Woodbridge, ON, Canada). Two drops (each containing 200 or 2000 IU of vitamin D) were taken orally once daily. Compliance was assessed by weighing bottles on a calibrated gravimetric scale at each study visit. Participants were not given calcium supplementation; however, they were given a document on lifestyle changes with advice on optimal calcium intake.

Participants were assigned to a vitamin D dose in accordance with the randomization schedule. Block randomization in units of 10 was done to confirm an equal distribution of vitamin D doses within sites. Participants and study staff were blinded to treatment allocation and to the results of any 25-hydroxyvitamin D test performed after the screening visit.

Blood Pressure Monitoring

Follow-up visits occurred every 2 months after the randomization visit until the end of the study. At every study visit, blood pressure was measured 4 times with a validated digital blood pressure monitor (HEM-907X, Omron Healthcare, Inc, Banncockburn, IL) and was averaged across the final 3 measurements. In addition, at baseline and 6 months, 24-hour ambulatory blood pressure data were collected with a 24-hour ambulatory monitor (Spacelabs Healthcare, Issaquah, WA) with an appropriately sized cuff. The protocol for clinic and 24-hour ambulatory blood pressure monitoring was standardized across all sites, and details are given in the online-only Data Supplement.

Monitoring

Blood samples were shipped to a central laboratory (Esoterix Clinical Laboratory Services, LabCorp, Cranford, NJ). Laboratory measurements were obtained every study visit and included plasma calcium, phosphorus, creatinine, aspartate and alanine aminotransferases, and serum 25-hydroxyvitamin D levels. We used a direct competitive chemiluminescence immunoassay (DiaSorin Inc, Stillwater, MN) for quantitative determination of total 25-hydroxyvitamin D in serum.[16] The intra-assay and interassay coefficients of variation were <5% and 10%, respectively (assay range, 4–150 ng/mL). Study data were reviewed by an external Data and Safety Monitor during and at the completion of the study.

End Points and Sample Size Estimates

The primary end point of the study was the change in mean 24-hour ambulatory systolic blood pressure. The secondary end points of the study included the change in mean 24-hour ambulatory diastolic blood pressure, daytime and nighttime ambulatory blood pressure, clinic blood pressure and pulse pressure, and the relation of vitamin D status to change in clinic and 24-hour ambulatory blood pressures.

Sample size estimates were based on data for the standard deviation of the change in 24-hour ambulatory systolic blood pressure from previous studies.[17] We powered the study to detect a 3-mm Hg difference in the primary end point. To achieve this power, we originally targeted a sample size of 450 randomized individuals. Because we enrolled a young and asymptomatic study population, we incorporated the assumption of a 20% dropout rate. With 20% dropout, we estimated that we would have 80% power to detect a 2.8-mm Hg difference in the primary end point. With twice the dropout rate, our minimum detectable difference for the primary end point would be only slightly higher, at 3.2 mm Hg between the 2 arms.

In September 2011, after 160 participants had been randomized, the investigators were notified by the Ddrops Co that random lot testing indicated that up to 40 participants in the high-dose arm had received a mean dose of 2000 rather than 4000 IU/d. After the Institutional Review Board and the US Food and Drug

Administration were informed, a new lot was established, and the vitamin D bottles for existing subjects were replaced. Blinding was maintained throughout the change. Concentration stability was confirmed during the remainder of the study. The target sample size was raised by 80 subjects, from 450 to 530 participants, to offset any potential reduction in power from the 40 participants in the high-dose arm who may have received the reduced dose. All analyses were conducted by the intention-to-treat principle, with planned secondary analyses stratified by vitamin D lot (eg, before or after September 2011).

Statistical Analyses

Demographics and baseline characteristics for randomized subjects were summarized by calculating median and interquartile range for continuous variables and percentages for categorical variables. The bivariate comparisons between groups were performed with the use of the Wilcoxon rank-sum test or χ^2 test. To model the change in mean 24-hour systolic blood pressure, we used an ordinary least-squares model that included treatment group as the main effect, with adjustment for race, study site, randomization season, and baseline blood pressure. A race-by-randomization interaction term was also included in the model to test whether treatment effects differed by race. In secondary analyses, we repeated the analysis by using the most recent clinic blood pressure to impute missing values for mean 24-hour ambulatory blood pressure. We fitted a generalized ordinary least-squares model for each secondary outcome variable, with randomization group, baseline blood pressure, study site, days from randomization, and a randomization group–by–days from randomization interaction term as covariates, along with a continuous autoregressive correlation structure to account for repeated measures for each study subject. The relation between blood pressure and total 25-hydroxyvitamin D in serum was also assessed with a nonparametric Spearman correlation coefficient. All analyses were performed with R version 3.0.1 statistical software.

Results

A total of 1343 individuals were screened for eligibility across all sites. We randomized 534 eligible individuals (Figure 1). The mean age was 36±10 years; 68% were men; and 54% were nonwhite. The mean 25-hydroxyvitamin D level was 15.7±6.3 ng/mL, with nearly three quarters of the study sample (73%) <20 ng/mL. There were no significant differences between the high-dose and low-dose vitamin D arms in any of the clinical or demographic characteristics (Table 1).

The initial clinic blood pressure was in the hypertensive range (≥140/90 mm Hg) for 28% of study participants. A 24-hour ambulatory blood pressure measurement was available for all subjects at baseline. As expected, mean ambulatory systolic and diastolic blood pressures were slightly lower than the corresponding clinic blood pressures, which was largely attributable to lower blood pressures at night (Table 1). Nonetheless, nearly half of the study sample met the definition of hypertension by 24-hour measurements.

Forty individuals (15%) in the high-dose arm and 58 individuals (21%) in the low-dose arm failed to complete the required 6-month follow-up. An additional 26 (11%) and 27 (10%) of individuals in each arm were withdrawn early by study investigators, with the most common reasons being noncompliance with study drug or elevated clinic blood pressure (Figure 1). Thus, 383 subjects (72%) completed the 6-month follow-up visits. Ten subjects in each arm were excluded from the final analysis because of incomplete 24-hour blood pressure data, leaving 188 subjects in the high-dose arm and 175 in the low-dose arm with complete data for the primary end point. Characteristics of these individuals are shown in Table I in the online-only Data Supplement and are similar to those in the randomized sample. A total of 455 subjects (85% of the randomized sample) completed at least 1 follow-up study visit with a clinic blood pressure measurement and are included in analyses of end points not requiring 24-hour blood pressure measurements.

Serum 25-hydroxvitamin D levels at each study visit are shown in Figure 2. At the 2-month visit, median 25-hydroxyvitamin D levels were 33 ng/mL (interquartile range, 26–40 ng/mL) in the high-dose arm versus 20 ng/mL (interquartile range, 15–25 ng/mL) in the low-dose arm (P<0.001). Levels remained at these levels in both study arms for the remainder of the

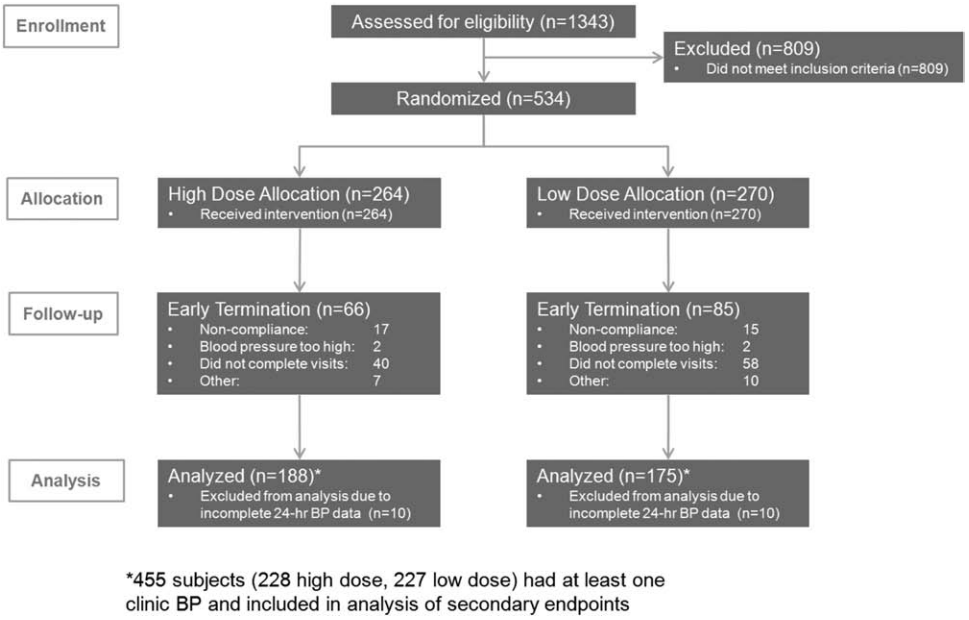

Figure 1. CONSORT (Consolidated Standards of Reporting Trials) diagram. BP indicates blood pressure.

Table 1. Baseline Characteristics of Randomized Participants

	High-Dose Arm (n=264)	Low-Dose Arm (n=270)
Age, y	37±10 (39, 28–45)	36±10 (36, 28–45)
Male, %	72	65
Body mass index, kg/m^2	28.1±5.9 (27, 24–32)	28.1±5.2 (28, 25–31)
25-Hydroxyvitamin D, ng/mL	15.6±6.5 (15, 11–21)	15.8±6.2 (15, 11–20)
Hypertension, %	49	46
White, %	44	47
Black, %	49	47
Other, %	8	6
Clinic systolic BP, mm Hg	131±10 (130, 125–136)	130±10 (129, 123–136)
Clinic diastolic BP, mm Hg	82±9 (82, 76–88)	81±9 (81, 75–87)
24-h systolic BP, mm Hg	127±10 (127, 120–134)	127±9 (127, 121–133)
24-h diastolic BP, mm Hg	78±8 (78, 73–83)	77±8 (77, 72–82)
Daytime systolic BP, mm Hg	130±10 (129, 122–137)	130±10 (130, 123–136)
Daytime diastolic BP, mm Hg	81±9 (81, 74–86)	80±9 (80, 73–85)
Nighttime systolic BP, mm Hg	121±12 (120, 112–128)	121±10 (120, 114–127)
Nighttime diastolic BP, mm Hg	71±10 (70, 64–78)	71±9 (70, 64–77)
Site, n (%)		
Boston	184 (70)	186 (69)
Hartford	28 (11)	31 (12)
Minneapolis, Allina Healthcare	36 (14)	38 (14)
Minneapolis, Cultural Wellness Center	16 (6)	15 (6)
Season of enrollment, %		
Winter	39	38
Spring	23	27
Summer	15	13
Fall	23	22

For continuous variables, values are mean±SD (median, interquartile range). Hypertension is defined on the baseline ambulatory BP monitoring status, that is, mean 24-hour systolic or diastolic BP ≥130/80 mm Hg or mean daytime systolic BP ≥135/85 mm Hg. BP indicates blood pressure.

6-month follow-up period. At the final visit, the proportions of individuals with 25-hydroxyvitamin D <20 ng/mL were 21% and 48% in the high-dose and low-dose arms, respectively.

Results for the primary end point are shown in Table 2. The change from baseline 24-hour systolic blood pressures did not differ (−0.8 versus −1.6 mm Hg in the high-dose and low-dose arms, respectively; P=0.71). At 6 months, the mean 24-hour systolic blood pressure was 126.5±10 mm Hg in the high-dose arm and 125.7±9 mm Hg in the low-dose arm (P=0.58). Similar results were obtained for 24-hour diastolic blood pressures (Table 2). As shown in Table 3, there were no significant differences in any of the other secondary blood pressure end points according to vitamin D assignment. Trends in clinic blood pressure across study visits are shown in Figure 3, which demonstrates no significant change in systolic or diastolic blood pressure in either study arm.

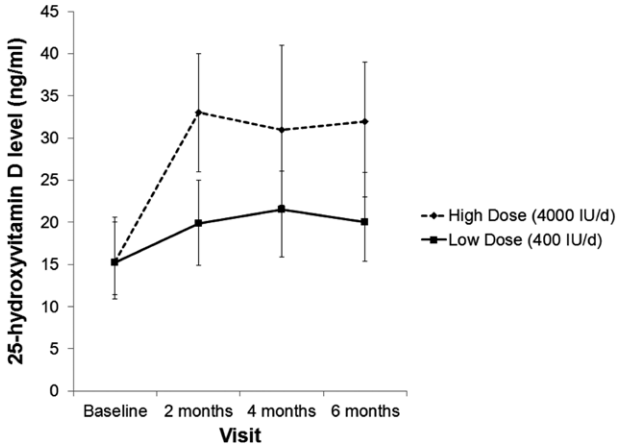

Figure 2. 25-Hydroxyvitamin D levels at each study visit according to treatment group.

We performed additional analyses to assess the relation between change in vitamin D levels and change in blood pressure. There was no association between the change in 25-hydroxyvitamin D and the change in the primary 24-hour blood pressure end point (Spearman coefficient, −0.05, P=0.34; Figure 4). Even among individuals with large increases in 25-hydroxyvitamin D during the study, there was no discernible trend toward lower 24-hour blood pressure. Figure I in the online-only Data Supplement depicts the relation between achieved vitamin D level at each study visit and clinic-measured systolic blood

Table 2. Results for 24-Hour Ambulatory Blood Pressure

	High-Dose Arm, mm Hg	Low-Dose Arm, mm Hg	*P* Value
At 6 mo			
Systolic BP	127±10 (125, 120 to 133)	126±9 (126, 119 to 132)	0.58
Diastolic BP	77±9 (76, 71 to 83)	76±8 (76, 72 to 81)	0.90
Change from baseline			
Systolic BP *	−0.8±8.7 (−1.2, −6 to 5)	−1.6±8.8 (−0.4, −7 to 4)	0.71
Diastolic BP	−1.2±6.5 (−1.5, −5 to 2)	−1.0±6.8 (−0.2, −5 to 4)	0.43

Values are mean±SD (median, interquartile range). BP indicates blood pressure.

*The primary end point is the change in 24-hour ambulatory BP between baseline and 6 months. For comparison of change in 24-hour ambulatory BPs, *P* values are calculated with a nonparametric Wilcoxon rank-sum test. For comparison of 6-month BPs, *P* values are based on ANCOVA, with race/ethnicity, study site, randomization season, baseline BP, and age by randomization group as covariates.

pressure, again showing no association, even among individuals who achieved high levels of 25-hydroxyvitamin D.

The results of prespecified subgroup analyses are shown in Figure 5 for the primary end point. These analyses revealed no evidence of heterogeneity in the study results. Analyses stratified by enrollment before or after September 2011 also showed no difference in the results. Finally, we repeated the analysis for the primary end point using clinic blood pressures to impute missing values for 24-hour ambulatory blood pressure at 6 months. This analysis yielded results similar to those of the primary analysis, with no significant difference in 24-hour systolic blood pressure between the high-dose and low-dose arms (*P*=0.99).

The mean drop use was 96% and 97% in the high-dose and low-dose vitamin D arms, respectively, on the basis of bottle weights. There was no significant difference in multivitamin use or body mass index between the high-dose and low-dose arms. Plasma calcium, creatinine, phosphorus, and transaminase levels did not differ between the high-dose and low-dose vitamin D arms at 6 months. Four individuals were noted to have an elevated calcium level (>10.5 mg/dL) during the study (3 in the high-dose arm, 1 in the low-dose arm). Two subjects (1 in each arm) were noted to have a phosphorus level >5 mg/dL. No serious adverse events were reported. The incidence of adverse events did not differ between the high-dose and low-dose arms (11 [4%] in the high-dose group versus 12 [4%] in the low-dose group). The most common events were headaches, nausea, cold, cough, insomnia, and fatigue. None of the adverse events were considered likely to be related to vitamin D supplementation.

Discussion

DAYLIGHT is the largest prospective, randomized trial to test the effect of vitamin D supplementation on blood pressure. We found no evidence that vitamin D supplementation lowered blood pressure in individuals with vitamin D deficiency and untreated prehypertension or stage 1 hypertension. This result was consistent across a range of blood pressure end points, including the primary end point of 24-hour ambulatory systolic blood pressure, and across multiple subgroups.

Despite the large body of observational evidence suggesting a link between vitamin D deficiency and hypertension, only a few prospective trials have addressed this question. Pfeifer and colleagues[13] randomized 148 postmenopausal women to 800 IU/d cholecalciferol and calcium versus calcium alone for 8 weeks. They observed a significant reduction in blood pressure in both arms but a greater decrease in systolic blood pressure in the vitamin D arm. Systolic blood pressure was higher at baseline in the vitamin D arm, raising the possibility that regression to the mean could have contributed to the findings. Two recent trials reported negative results. Larsen and colleagues[11] studied 112 hypertensive patients randomized to 3000 IU/d cholecalciferol versus placebo for 5 months. They found no significant difference in 24-hour blood pressure between the treatment groups, although there was a reduction in a secondary end point (clinic systolic blood pressure;

Table 3. Results for Secondary End Points at 6 Months

	High-Dose Arm (n=188), mm Hg	Low-Dose Arm (n=175), mm Hg	*P* Value*
Clinic systolic BP	128±12 (127, 120–136)	127±11 (125, 120–133)	0.88
Clinic diastolic BP	83±10 (83, 76–89)	82±9 (81, 76–87)	0.81
Daytime systolic BP	129±10 (128, 122–137)	128±9 (128, 122–135)	0.54
Daytime diastolic BP	80±9 (79, 74–85)	79±8 (79, 74–84)	0.82
Nighttime systolic BP	120±12 (118, 112–126)	119±10 (118, 113–126)	0.33
Nighttime diastolic BP	70±10 (69, 63–76)	70±9 (70, 64–75)	0.59

Values are mean±SD (median, interquartile range). BP indicates blood pressure.

*For clinic BPs, *P* values are based on generalized ordinary least-squares regression with the following covariates: baseline BP, study site, days from randomization, randomization arm, and randomization arm–by–days from randomization interaction. For daytime and nighttime BPs, *P* values are based on ANCOVA, adjusted for race/ethnicity, study site, randomization season, and baseline BP. A race-by-randomization group term was also included in the ANCOVA model.

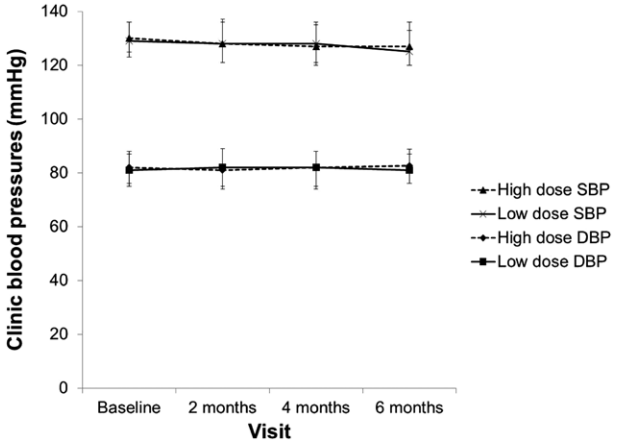

Figure 3. Clinic systolic (SBP) and diastolic (DBP) blood pressures at each study visit according to treatment group.

P=0.02). Witham and colleagues[12] performed a randomized trial of 159 elderly individuals (mean age, 77 years) assigned to 100 000 IU of cholecalciferol every 3 months versus placebo for 1 year. No significant differences were noted for 24-hour blood pressure, clinic blood pressure, or endothelial function. Notably, DAYLIGHT randomized more participants than all 3 previous trials combined.

Recently, Vimaleswaran and colleagues[5] used a mendelian randomization approach to test whether vitamin D–related polymorphisms were related to blood pressure. They derived 2 "genetic scores" for 25-hydroxyvitamin D levels based on genes involved in the synthesis or metabolism of vitamin D. They found that the synthesis variants, but not the metabolism variants, were associated with blood pressure (*P*=0.0498 for systolic and *P*=0.01 for diastolic blood pressure). Instrumental variables analyses suggested that each 10% increase in circulating 25-hydroxyvitamin D levels would lead to a 0.37-mm Hg reduction in systolic blood pressure; a doubling of 25-hydroxyvitamin D levels, as observed in the high-dose arm of DAYLIGHT, would be predicted to reduce systolic blood pressure by 4 to 5 mm Hg. There was heterogeneity among the studies included in the Vimaleswaran et al[5] meta-analysis, with only 1 of >30 cohorts showing a statistically significant association between synthesis variants and systolic blood pressure.

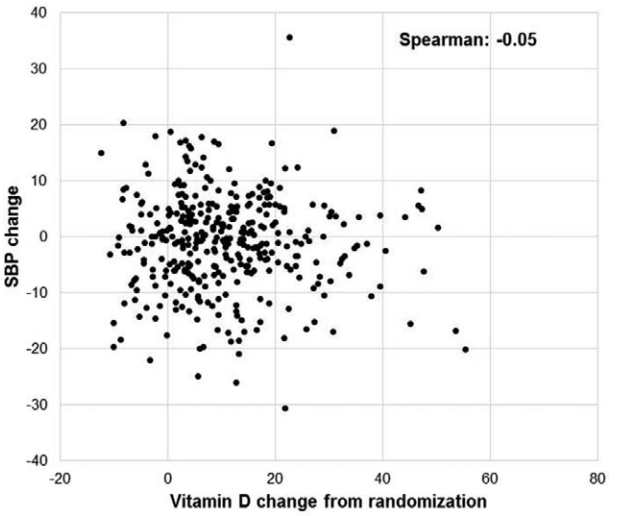

Figure 4. Relation of change in 24-hour mean systolic blood pressure (SBP) and change in 25-hydroxyvitamin D level between baseline and 6 months.

The prior studies motivated the conduct of a well-powered randomized trial to assess whether vitamin D supplementation reduces blood pressure. We targeted our intervention to individuals with documented low vitamin D status because such individuals are most likely to benefit from vitamin D supplementation. The median 25-hydroxyvitamin D level at baseline was 15.3 ng/mL, lower than most thresholds for defining vitamin D deficiency. All subjects in DAYLIGHT had a baseline 25-hydroxyvitamin D level of 25 ≤ng/mL, and nearly three quarters had a level <20 ng/mL. In contrast, prior trials often included individuals with 25-hydroxyvitamin D levels >30 ng/mL.[9]

Furthermore, we focused on individuals with untreated prehypertension or stage 1 hypertension. Experimental studies suggest that the effect of vitamin D on blood pressure may be blunted by antihypertensive therapy, particularly agents that block the renin-angiotensin system.[18] The study was designed to minimize confounding by concomitant medications in a sample in which nearly a third of individuals had initial blood pressures in the hypertensive range. The exclusion of individuals on antihypertensive therapy distinguishes DAYLIGHT from other vitamin D supplementation/blood pressure trials in which a large proportion of subjects were on antihypertensive treatment at baseline. For instance, in the trial of Witham and colleagues,[12] subjects were taking a median of 2 antihypertensive medications, and >40% were on an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker.

DAYLIGHT subjects in the high-dose arm experienced a >2-fold increase in 25-hydroxyvitamin D levels. By the end of the trial, the median 25-hydroxyvitamin D level in the high-dose arm exceeded 30 ng/mL, indicating that the majority of subjects were vitamin D "replete" according to conventional definitions. Notably, there was substantial interindividual variation in the increase in 25-hydroxyvitamin D in response to vitamin D supplementation. Some subjects with a lower-than-expected response may have been noncompliant, although we performed regular compliance assessment with a gravimetric scale. Prior pharmacokinetic and genetic studies suggest that biological factors may play an important role in determining response to supplementation.[19,20] In addition, although some studies using 4000 IU/d cholecalciferol have found larger mean increases in 25-hydroxyvitamin D levels,[10,21] those studies focused on different populations and used supplements in pill rather than liquid form. Whether the formulation of cholecalciferol influences bioavailability is not well established.

Although we cannot exclude the possibility that even higher levels of vitamin D than those achieved in DAYLIGHT would be needed to affect blood pressure, there is no biological basis for postulating such a threshold effect. In addition, the observational studies that motivated DAYLIGHT document a linear relationship with blood pressure across the range of vitamin D levels observed in the trial.[4] Finally, even in the subset of individuals who attained 25-hydroxvitamin D levels >50 ng/mL, there was no evidence of a trend toward lower blood pressures (Figure I in the online-only Data Supplement).

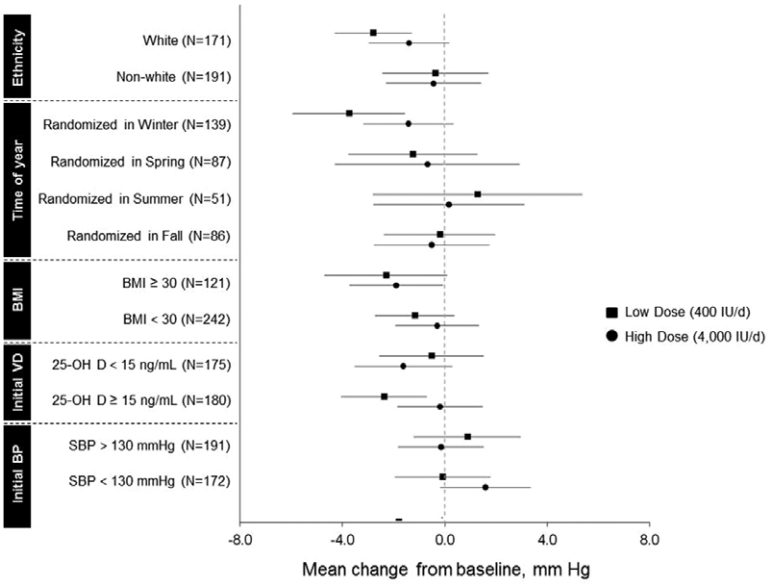

Figure 5. Change in mean 24-hour systolic blood pressure (SBP) between baseline and 6 months in prespecified subgroups. BMI indicates body mass index; and VD, vitamin D.

Approximately 20% of participants failed to complete the required follow-up visits, and an additional 10% were withdrawn early for meeting one of the exclusion criteria. High dropout rates are frequently seen in trials of vitamins or supplements. For instance, in the recently completed Trial to Assess Chelation Therapy (TACT) trial, 46% of subjects discontinued their multivitamins, with the most commonly cited reason being lack of interest in continuing vitamin therapy.[22] Adherence to a study medication may be particularly challenging for individuals who are young and asymptomatic such as those enrolled in DAYLIGHT.

Because the likelihood of dropout was incorporated into the original power estimates, subject dropout had only a marginal impact on the final statistical power. The trial was designed to detect a difference in the primary end point of 3 mm Hg with 80% power. Post hoc calculations using the final sample size indicate a detectable difference of 3.1 mm Hg with 80% power. Furthermore, there was no evidence that individuals who completed the study differed from those who did not with regard to baseline blood pressure and demographic characteristics. It is also noteworthy that analyses of clinic blood pressure, which included up to 455 subjects and provided even greater statistical power, yielded findings very consistent with those of the primary analyses.

Several limitations deserve comment. Recently, Powe and colleagues[23] reported that the degree of vitamin D deficiency in blacks may be overstated because of lower vitamin D–binding protein concentrations, leading to greater bioavailability at lower 25-hydroxyvitamin D levels. Concentrations of vitamin D binding protein are largely determined by race and genotype. We did not incorporate vitamin D–binding protein measurements or genotyping in our enrollment criteria to assess vitamin D status because DAYLIGHT was initiated before the publication of the Powe et al study. Nonetheless, our findings were nearly identical in blacks and whites and consistent across the full range of baseline 25-hydroxyvitamin D levels included in the study, suggesting that vitamin D has neutral effects on blood pressure regardless of race or baseline vitamin D status.

As with all randomized trials, the generalizability of our results to populations not studied is uncertain. For instance, we cannot exclude the possibility that vitamin D supplementation would have been more effective in individuals with greater degrees of hypertension or on baseline antihypertensive therapy. Nonetheless, there is no evidence from experimental or epidemiological studies to suggest a mechanism by which vitamin D supplementation would be effective only in the context of existing antihypertensive medications. Nearly a third of our subjects had baseline blood pressures in the hypertensive range, and the remaining two thirds were prehypertensive, a group with high rates of progression to overt hypertension and increased cardiovascular risk.[24,25]

It is possible that concomitant calcium supplementation may be required to see antihypertensive effects from vitamin D therapy. We delivered a standard set of dietary guidelines to participants in both arms that included recommendations on calcium intake. Calcium may itself have antihypertensive effects. A trial by Pfeifer and colleagues[13] suggested that vitamin D may potentiate the blood pressure effects of calcium, but this finding has not been replicated in other larger studies of vitamin D and calcium supplementation.[12,14] Furthermore, experimental studies indicate that the putative vascular effects of vitamin D are not calcium dependent.[18] Indeed, results of recent meta-analyses raise the possibility that calcium supplementation may increase cardiovascular risk,[26] a controversy likely to discourage the routine inclusion of calcium supplements in randomized trials with vitamin D.

We did not include a placebo arm in this trial, instead administering 400 IU/D cholecalciferol to subjects in the control arm, that is, equivalent to the amount of vitamin D found in a typical multivitamin. During the design of DAYLIGHT, the Institutes of Medicine released guidelines on the recommended dietary intakes of vitamin D, which was 600 IU/D (from all sources) for the age group included in the trial. Because vitamin D deficiency was an inclusion criterion for DAYLIGHT, the investigators felt that it would be difficult

to justify omission of vitamin D supplementation entirely from the control arm, particularly because participants were discouraged from taking out-of-study supplementation during the trial.

We cannot exclude the possibility that the vitamin D preparation in the low-dose arm had modest effects on blood pressure, attenuating our ability to detect a difference in the overall end point. Nonetheless, we observed minimal to no change in blood pressure in the high-dose arm when considered by itself, making it very unlikely that use of a placebo arm would have led to a different result. The high-dose regimen in DAYLIGHT was selected with the goal of achieving vitamin D "sufficiency" in the majority of participants, in contrast to the 10-fold lower dose in the control arm. Accordingly, by the end of the study, nearly 80% of individuals in the low-dose arm continued to have 25-hydroxyvitamin D levels <25 ng/mL. Despite the between-group contrast in vitamin D levels achieved by the end of the study, there was no subgroup for which the change in blood pressure was larger in the high-dose group than in the low-dose group.

Although we cannot draw conclusions about longer periods of vitamin D supplementation (>6 months), vitamin D status improved within 2 months of starting high-dose vitamin D and plateaued thereafter. Blood pressure is a physiological end point that typically responds rapidly to intervention. Furthermore, although DAYLIGHT is the largest prospective study of vitamin D supplementation and blood pressure, we cannot exclude small changes in blood pressure (1–2 mm Hg) resulting from the intervention. Much larger studies would be required to detect changes in this range. Notably, the absolute changes in mean 24-hour systolic blood pressure were greater in the low-dose arm than in the high-dose arm (−1.6 versus −0.8 mm Hg).

Finally, our findings do not exclude the possibility that vitamin D supplementation may be beneficial for other cardiovascular end points. Results of ongoing trials such as the Vitamin D and OmegA-3 Trial (VITAL) study should provide further information on whether vitamin D supplementation has a favorable effect on overall cardiovascular risk.[27,28]

Conclusions

Vitamin D supplementation did not reduce blood pressure in individuals with prehypertension or stage 1 hypertension and vitamin D deficiency. Added to the existing body of evidence from smaller randomized trials, our findings suggest that the association between vitamin D status and hypertension noted in observational studies is not causal.

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Disclosures

All authors have completed and submitted the *International Committee of Medical Journal Editors* Form for Disclosure of Potential Conflicts of Interest. Dr Wang reports research support and consultant fees from DiaSorin Inc. Dr Valcour is an employee of LabCorp Inc. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Vitamin D deficiency and hypertension are disorders with a high prevalence worldwide. A large body of observational and experimental evidence suggests a link between vitamin D deficiency and elevated blood pressure, but data from prospective studies are limited. We conducted a randomized, double-blind, controlled, clinical trial in vitamin D–deficient individuals (mean 25-hydroxyvitamin D level, 15.3 ng/mL) with untreated prehypertension or stage 1 hypertension at 4 sites in the United States. We randomized 534 participants to 6 months of high-dose (4000 IU/d) versus low-dose (400 IU/d) vitamin D supplementation. The primary end point was change in mean 24-hour systolic blood pressure. At the end of the study, there was no significant difference in the primary end point (−0.8 versus −1.6 mm Hg in the high-dose and low-dose arms, respectively; P=0.71) or in any of the secondary end points. Furthermore, there was no evidence of association between change in 25-hydroxyvitamin D and change in 24-hour systolic blood pressure at 6 months (P=0.34). The results were consistent across prespecified subgroups, including in black participants. Our findings suggest that the association between vitamin D status and elevated blood pressure noted in observational studies is not causal.

Vitamin D Therapy in Individuals With Prehypertension or Hypertension: The DAYLIGHT Trial

Pankaj Arora, Yanna Song, Jeffery Dusek, Gregory Plotnikoff, Marc S. Sabatine, Susan Cheng, Andre Valcour, Heather Swales, Beth Taylor, Erin Carney, Derek Guanaga, Joseph R. Young, Courtney Karol, Michael Torre, Atum Azzahir, Semerit M. Strachan, Dillon C. O'Neill, Myles Wolf, Frank Harrell, Christopher Newton-Cheh and Thomas J. Wang

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SUPPLEMENTAL MATERIAL

SUPPLEMENTARY METHODS

Full list of exclusion criteria

Individuals were excluded if they had any of the following: use of any anti-hypertensive medication in the past 3 months or anticipated or planned use in the next 6 months; use of vitamin D supplementation in the last 3 months, defined as vitamin D found in a multivitamin or supplement totaling >400 IU per day, or anticipated or planned use in the next 6 months; use of St. John's wart, rifampin, any treatment for HIV, orlistat, oral glucocorticoids, phenobarbital, phenytoin, mineral oil, or bile acid sequestrants in the last 3 months or anticipated or planned use in the next 6 months; history of diabetes mellitus (including Type 1, Type 2 and diet controlled); calcium >10.5 mg/dl or phosphorus >5 mg/dl; women who were pregnant, nursing, or of childbearing potential or planning or anticipating pregnancy in next 6 months; serum creatinine >2.0 mg/dl or estimated glomerular filtration rate <30 ml/min; history of kidney stones; body mass index >38 kg/m^2; known cardiovascular disease, defined as prior myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass or stroke; history of cirrhosis or severe liver disease (defined as history of gastrointestinal bleeding from liver disease, jaundice or ascites); current heavy alcohol use: defined as drinking 5 or more drinks per occasion on 5 or more days in the past 30 days; history of ulcerative colitis, Crohn's disease, celiac disease, colostomy, pancreatic enzyme deficiency, short bowel syndrome, gastric bypass, cystic fibrosis, or dumping syndrome; allergy to coconut; regular use or planned use of artificial tanning lights in the next 6 months; use of any investigational product or device in the last 3 months or planned use in the next 6 months; unwillingness or inability to comply with study requirements; or inability to provide informed consent.

Blood pressure monitoring

Clinic blood pressure was measured three times in the non-dominant arm with a validated digital blood pressure monitor (HEM-907X [Omron Healthcare, Inc, Banncockburn, IL]) and appropriately sized cuff. The average systolic and diastolic blood pressure was calculated from the last two measurements. Ambulatory blood pressure measurements were taken every 20 minutes from 0600-2200 h and every 30 minutes from 2200-0600 h. Daytime was defined as 0600-2159 h and nighttime was defined as 2200-0559 h. Thus, the maximum total number of analyzable measurements was 64, with 48 daytime and 16 nighttime measurements. The same ambulatory blood pressure device was used at baseline and follow up for each study participant. If fewer than two-thirds of daytime (<33 measurements) or nighttime (<11 measurements) measurements were accurately recorded, the subject was asked to repeat the 24-hour monitoring procedure. Clinic and ambulatory blood pressure measurements were done in accordance with the American Heart Association guidelines.[1, 2]

Early termination

Participants were discontinued from the protocol if they developed hypercalcemia (calcium > 10.5 mg/dl) or hyperphosphatemia (phosphorus > 5 mg/dl), or if 25-hydroxyvitamin D levels exceeded 100 ng/ml. Other reasons for early termination included failure to complete required visits, non-compliance with study medication (as defined by estimated drops dispensed < 80% or >120% of the target), or elevated blood pressure. The thresholds for early termination due to blood pressure were a systolic > 159 mm Hg or diastolic > 99 mm Hg at 2 consecutive study visits, or systolic > 169 mm Hg or diastolic > 109 mm Hg at any study visit. In addition, if anti-

hypertensive therapy was initiated for any reason, then subjects were discontinued from the study.

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FIGURE LEGENDS

Supplementary Figure 1: Relation of clinic systolic blood pressure (SBP) and 25-hydroxyvitamin D level at each study visit in all available subjects

Supplementary Table 1. Participants with and without complete blood pressure follow-up

	Subjects with full follow-up blood pressure data (N=363)	Subjects without full follow-up blood pressure data (N=171)	All Subjects (N=534)
Age, years	37 ± 9 (38, 28 - 46)	35 ± 10 (36, 27 - 44)	36 ± 10 (38, 28 - 45)
Male	70%	64%	68%
Body mass index, kg/m2	28.1 ± 5.2 (28, 24 - 31)	28.1 ± 6.3 (27, 24 - 32)	28.1 ± 5.6 (28, 24 - 31)
25-OH vitamin D, ng/ml	15.5 ± 6.3 (15, 11 - 20)	16.2 ± 6.4 (15, 12 - 21)	15.7 ± 6.3 (15, 11 - 20)
White	47%	42%	46%
Black	46%	51%	48%
Other	6%	7%	7%
Clinic systolic BP, mm Hg	131 ± 9 (130, 124 - 136)	129 ± 11 (128, 123 - 136)	130 ± 10 (129, 123 - 136)
Clinic diastolic BP, mm Hg	82 ± 9 (82, 77 - 88)	80 ± 9 (80, 75 - 87)	81 ± 9 (81, 76 - 88)
24-hour systolic BP, mm Hg	127 ± 9 (127, 121 - 133)	127 ± 11 (127, 120 - 134)	127 ± 10 (127, 121 - 133)
24-hour diastolic BP, mm Hg	78 ± 8 (77, 72 - 83)	78 ± 8 (78, 72 - 83)	78 ± 8 (78, 72 - 83)
Season of enrollment %			
Winter	38%	39%	39%
Spring	24%	28%	25%
Summer	14%	15%	14%
Fall	24%	19%	22%

For continuous variables, values are mean ± SD (median, IQR); SD: standard deviation; IQR: interquartile range; BP: blood pressure.

Supplementary Figure 1