Vitamin D Therapy in Individuals with Pre-Hypertension or Hypertension:  

The DAYLIGHT Trial

Running title: Arora et al.; Vitamin D supplementation and blood pressure

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Abstract

**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 aged 18 to 50 years with low vitamin D status (25-hydroxyvitamin D levels ≤ 25 ng/ml) and systolic blood pressure 120-159 mm Hg. Participants were randomized to high-dose (4,000 IU/day) versus low-dose (400 IU/day) oral vitamin D3 for 6 months. The primary endpoint was change in mean 24-hour systolic blood pressure. Secondary endpoints 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 one 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 endpoint (change in mean 24-hour systolic blood pressure, -0.8 mm Hg versus -1.6 mm Hg in the high-dose and low-dose arms, p=0.71) or in any of the secondary endpoints. Further, 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 pre-specified subgroups.

**Conclusions**—Vitamin D supplementation did not reduce blood pressure in individuals with pre- 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 Information**—ClinicalTrials.gov. Identifier: NCT01240512.

**Key words:** vitamin D, blood pressure, blood pressure measurement/monitoring, hypertension, high blood pressure
Background

Vitamin D deficiency is a common problem with implications for human health. A large body of epidemiological evidence links vitamin D deficiency with a higher risk of cardiovascular disorders including hypertension. A meta-analysis of observational studies found that every 16 ng/ml decrement in vitamin D was associated with a 16% higher risk of hypertension. A meta-analysis of population genetic studies suggested that polymorphisms related to lower vitamin D status were associated with higher blood pressure. Additionally, low vitamin D concentrations have been shown to predict future hypertension among individuals with normal blood pressure at baseline. 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. Disruption of these receptors in animals is associated with elevated blood pressure, which can be normalized with vitamin D administration.

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. In most of the trials, blood pressure was not the primary endpoint nor was it measured using standardized protocols. These trials also typically randomized fewer than 150 participants and included a large proportion of individuals who were already on anti-hypertensive therapy. Importantly, very few non-white 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. Accordingly, we
conducted the DAYLIGHT study, 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 (4,000 IU per day) versus low (400 IU/ day) dose vitamin D supplementation in individuals with pre-hypertension and untreated stage 1 hypertension and vitamin D deficiency. Participants were recruited at 4 sites (the Massachusetts General Hospital [Boston, MA], Hartford Hospital [Hartford, CT], the 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 less than 99 mm Hg at two clinic visits. The other main inclusion criterion was a 25-hydroxyvitamin D level of 25 ng/ml or below at the screening visit.

Individuals were excluded if they had use of any anti-hypertensive medication in the past 3 months, use of vitamin D supplementation in the past 3 months (defined as vitamin D found in a multivitamin or supplement totaling >400 IU per day), or known cardiovascular disease
(defined as prior myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass or stroke). Other exclusion criteria are detailed in the Supplementary Material.

**Vitamin D supplementation**

Vitamin D was administered using once-daily oral doses of vitamin D₃ (cholecalciferol), with a total of 4,000 IU or 400 IU, in the high-dose and low-dose arms, respectively. Administration was via gravity-metered dropper bottles to deliver a consistent dosage (D Drops, Canada). Two drops (each containing 200 IU 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 lifestyle changes document 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 using a validated digital blood pressure monitor (HEM-907X [Omron Healthcare, Inc, Bannockburn, IL]) and was averaged across the final 3 measurements. In addition, at baseline and 6 months, 24-hour ambulatory blood pressure data was collected using 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 in the Supplementary Material.
Material.

Monitoring

Blood samples were shipped to a central laboratory (Esoterix Clinical Lab 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. The intra and interassay coefficient of variation was less than 5 and 10 percent 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.

Endpoints and sample size estimates

The primary end point of the study was the change in mean 24-hour ambulatory systolic blood pressure. The secondary endpoints 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. We powered the study to detect a 3 mm Hg difference in the primary endpoint. In order 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 endpoint. With twice the dropout rate, our minimum detectable
difference for the primary endpoint would be only slightly higher, at 3.2 mm Hg between the two arms.

In September 2011, after 160 participants had been randomized, the investigators were notified by the D Drops Company that random lot testing indicated that up to 40 participants in the high-dose arm had received a mean dose of 2,000 IU per day, rather than 4,000 IU per day. After informing the Institutional Review Board and the FDA, a new lot was established, and 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, in order 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 (e.g. before or after September 2011).

Statistical Analyses
Demographics and baseline characteristics for randomized subjects were summarized by calculating median and inter-quartile range (IQR) 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 chi-square 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 fit a generalized ordinary least square 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 using statistical software R version 3.0.1.

Results

A total of 1,343 individuals were screened for eligibility across all sites. We randomized 534 eligible individuals (Figure 1). The mean age was $36 \pm 10$ years, 62% were men, and 56% were non-white. The mean 25-hydroxyvitamin D level was $15.7 \pm 6.3$ ng/ml, with nearly three-quarters of the study sample (73%) below $20$ 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 ($\geq 140/90$ mm Hg) for 28% of study participants. A 24-hour ambulatory blood pressure measurement was available on 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).

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 non-compliance 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 due to 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 endpoint. Characteristics of these individuals are shown in Supplementary Table 1, and are similar to those in the randomized sample. A total of 455 subjects (85% of the randomized sample) completed at least one follow-up study visit with a clinic blood pressure measurement, and are included in analyses of endpoints 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 (IQR, 26 to 40) in the high-dose arm versus 20 ng/ml (IQR, 15 to 25) in the low-dose arm (p<0.001). Levels remained at these levels in both study arms for the remainder of the 6-month follow-up period. At the final visit, the proportion 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 endpoint are shown in Table 2. The change from baseline 24-hour systolic blood pressures did not differ (-0.8 mm Hg vs -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 endpoints 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.
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 endpoint (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. Supplementary Figure 1 depicts the relation between achieved vitamin D level at each study visit and clinic-measured systolic blood pressure, again showing no association, even among individuals who achieved high levels of 25-hydroxyvitamin D.

The results of pre-specified subgroup analyses are shown in Figure 5 for the primary endpoint. 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. Lastly, we repeated the analysis for the primary endpoint using clinic blood pressures to impute missing values for 24-hour ambulatory blood pressure at 6 months. This analysis yielded similar results to 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 usage was 96% and 97% in the high-dose and low-dose vitamin D arms, respectively, based on 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. There were no serious adverse events 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 pre- or stage 1 hypertension. This result was consistent across a range of blood pressure endpoints, including the primary endpoint 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 randomized 148 post-menopausal women to 800 IU per day of cholecalciferol and calcium versus calcium alone for 8 weeks. They observed a significant reduction in blood pressure in both arms, but with a greater fall 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 studied 112 hypertensive patients randomized to 3,000 IU cholecalciferol per day versus placebo for 5 months. They found no significant difference in 24-hour blood pressure between treatment groups, though there was a reduction in a secondary endpoint (clinic systolic blood pressure, p=0.02). Witham and colleagues 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.12 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 colleagues5 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, respectively. 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 substantial heterogeneity among the studies included in the Vimaleswaran meta-analysis, with only 1 of more than 30 cohorts showing a statistically significant association between “synthesis” variants and systolic blood pressure. Further, the cohorts were roughly evenly split between those showing positive and negative associations between vitamin D status and systolic blood pressure.

The prior studies motivate the conduct of well-powered randomized trials to assess whether vitamin D supplementation reduces blood pressure. We targeted our intervention at 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 of below, and nearly three-
quarters had a level below 20 ng/ml. In contrast, prior trials often included individuals with 25-hydroxyvitamin D levels above 30 ng/ml.\textsuperscript{9}

Furthermore, we focused on individuals with untreated pre-hypertension or stage I hypertension. Experimental studies suggest that the effect of vitamin D on blood pressure may be blunted by anti-hypertensive therapy, particularly agents that block the renin-angiotensin system.\textsuperscript{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 anti-hypertensive therapy distinguishes DAYLIGHT from other vitamin D supplementation/blood pressure trials, in which a large proportion of subjects were on anti-hypertensive treatment at baseline. For instance, in the trial of Witham and colleagues, subjects were taking a median of 2 anti-hypertensive medications, and more than 40\% were on an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker.\textsuperscript{12}

DAYLIGHT subjects in the high-dose arm experienced more than 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” using conventional definitions. Notably, there was substantial inter-individual variation in the increase in 25-hydroxyvitamin D in response to vitamin D supplementation. Some subjects with less than expected response may have been noncompliant, though 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.\textsuperscript{19,20} Also, although some studies using 4,000 IU per day of cholecalciferol have found larger mean increases in 25-hydroxyvitamin D levels,\textsuperscript{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.

Though we cannot exclude the possibility that even higher levels of vitamin D than those achieved in DAYLIGHT would be needed to impact 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.\(^4\) Lastly, even in the subset of individuals who attained 25-hydroxvitamin D levels above 50 ng/ml, there was no evidence of a trend toward lower blood pressures (Supplementary Figure 1).

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 TACT trial, 46% of subjects discontinued their multivitamin, 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 in 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 endpoint 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 or 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 very consistent findings to those of the primary
Several limitations deserve comment. Recently, Powe and colleagues reported that the degree of vitamin D deficiency in blacks may be overstated due to 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 prior to the publication of the Powe 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 irrespective 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 anti-hypertensive therapy. Nonetheless, there is no evidence from experimental or epidemiologic studies to suggest a mechanism by which vitamin D supplementation would only be effective in the context of existing anti-hypertensive medications. Nearly a third of our subjects had baseline blood pressures in the hypertensive range, and the remaining two-thirds were “pre-hypertensive,” a group with high rates of progression to overt hypertension and increased cardiovascular risk.

It is possible that concomitant calcium supplementation may be required to see anti-hypertensive effects from vitamin D therapy. We delivered a standard set of dietary guidelines to participants in both arms, which included recommendations regarding calcium intake.
Calcium may itself have anti-hypertensive effects. A trial by Pfeifer and colleagues 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. Furthermore, experimental studies indicate that the putative vascular effects of vitamin D are not calcium-dependent. Indeed, results of recent meta-analyses raise the possibility that calcium supplementation may increase cardiovascular risk, 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 of cholecalciferol per day to subjects in the control arm, e.g. equivalent to the amount of vitamin D found in a typical multivitamin. During the design of DAYLIGHT, the Institutes of Medicine released guidelines regarding the recommended dietary intakes of vitamin D, which was 600 IU per day (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 as 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 endpoint. 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 below 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 physiologic endpoint that typically responds rapidly to intervention. Further, although DAYLIGHT is the largest prospective study of vitamin D supplementation and blood pressure, we cannot exclude small changes in blood pressure (1 to 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).

Lastly, our findings do not exclude the possibility that vitamin D supplementation may be beneficial for other cardiovascular endpoints. Results of ongoing trials, such as the VITAL study, should provide further information on whether vitamin D supplementation has a favorable effect on overall cardiovascular risk.27

In summary, vitamin D supplementation did not reduce blood pressure in individuals with pre-hypertension or stage I 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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the accuracy of the data analysis. Study concept and design: Dr. Arora, Dr. Swales, Azzahir, Dr. Strachan, Dr. Plotnikoff, Dr. Dusek, Dr. Wolf, Dr. Newton-Cheh and Dr. Wang. Acquisition of data: Carney, Guanaga, Karol, Torre, Young, Dr. Dusek, Azzahir, Dr. Strachan, Dr. Taylor, and Dr. Arora. Drafting of the manuscript: Dr. Arora, Dr. Newton-Cheh, and Dr. Wang. Critical revision of the manuscript for important intellectual content: Dr. Sabatine, Dr. Wolf, Dr. Cheng, Dr. Taylor, Dr. Swales, Dr. Song, and Dr. Harrell. Statistical analysis and interpretation of the data: Song, Dr. Harrell, Dr. Arora, Dr. Newton-Cheh, and Dr. Wang. Obtained funding: Dr. Wang. Administrative, technical, or material support: O’Neill, Carney, Guanaga, Azzahir, Dr. Strachan, and Dr. Valcour. Study supervision: Dr. Newton-Cheh and Dr. Wang.

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**References:**


5. Vimaleswaran KS, Cavadino A, Berry DJ, Jorde R, Dieffenbach AK, Lu C, Alves AC,
Heerspink HJ, Tikkanen E, Eriksson J, Wong A, Mangino M, Jablonski KA, Norle IM, Houston
DK, Abluwalia TS, van der Most PJ, Pasko D, Zgaga L, Thiering E, Vitart V, Fraser RM,
Huffman JE, de Boer RA, Schottker B, Saum KU, McCarthy MI, Dupuis J, Herzig KH, Sebert S,
Pouta A, Latinen J, Kleber ME, Navis G, Lorentzon M, Jameson K, Arden N, Cooper JA,
Acharya J, Hardy R, Raitakari O, Ripatti S, Billings LK, Lahti J, Osmond C, Penninx BW,
Reijnmark L, Lohman KK, Paternoster L, Stolk RP, Hernandez DG, Byberg L, Hagstrom E,
Melhus H, Ingelsson E, Mellstrom D, Ljunggren O, Tzoulaki I, MacLachlan S, Theodoratou E,
Tiesler CM, Jula A, Navarro P, Wright AF, Polasek O, Wilson JF, Rudan I, Salomaa V, Heinrich
J, Campbell H, Price JF, Karlsson M, Lind L, Michaelsson K, Bandinelli S, Frayling TM,
Hartman CA, Sorensen TI, Kritchevsky SB, Langdahl BL, Eriksson JG, Florez JC, Spector TD,
Lehtimaki T, Kuh D, Humphries SE, Cooper C, Oxlsson C, Marz W, de Borst MH, Kumari M,
AD, Pilz S, Whittaker JC, Jarvelin MR, Hypponen E. Association of vitamin d status with
arterial blood pressure and hypertension risk: A mendelian randomisation study. *Lancet Diabetes

25-hydroxyvitamin d3-1alpha-hydroxylase is expressed in human vascular smooth muscle
cells and is upregulated by parathyroid hormone and estrogenic compounds. *Circulation.*

EW, Ritz E. Identification and regulation of 1,25-dihydroxyvitamin d3 receptor activity and
biosynthesis of 1,25-dihydroxyvitamin d3. Studies in cultured bovine aortic endothelial cells

8. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-dihydroxyvitamin d(3) is a negative


10. Forman JP, Scott JB, Ng K, Drake BF, Suarez EG, Hayden DL, Bennett GG, Chandler PD,
Holllis BW, Emmons KM, Giovannucci EL, Fuchs CS, Chan AT. Effect of vitamin d

11. Larsen T, Mose FH, Bech JN, Hansen AB, Pedersen EB. Effect of cholecalciferol
supplementation during winter months in patients with hypertension: A randomized, placebo-

12. Witham MD, Price RJ, Struthers AD, Donnan PT, Messow CM, Ford I, McMurdie ME.
Cholecalciferol treatment to reduce blood pressure in older patients with isolated systolic

d(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. *J Clin Endocrinol Metab.* 2001;86:1633-1637.


**Table 1.** Baseline characteristics of randomized participants.

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<th>High-dose arm (N=264)</th>
<th>Low-dose arm (N=270)</th>
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<td><strong>Age, years</strong></td>
<td>37 ± 10 (39, 28 - 45)</td>
<td>36 ± 10 (36, 28 - 45)</td>
</tr>
<tr>
<td><strong>Male, %</strong></td>
<td>72%</td>
<td>65%</td>
</tr>
<tr>
<td><strong>Body mass index, kg/m²</strong></td>
<td>28.1 ± 5.9 (27, 24 - 32)</td>
<td>28.1 ± 5.2 (28, 25 - 31)</td>
</tr>
<tr>
<td><strong>25-OH vitamin D, ng/ml</strong></td>
<td>15.6 ± 6.5 (15, 11 - 21)</td>
<td>15.8 ± 6.2 (15, 11 - 20)</td>
</tr>
<tr>
<td><strong>Hypertensives, %</strong></td>
<td>49%</td>
<td>46%</td>
</tr>
<tr>
<td><strong>White, %</strong></td>
<td>44%</td>
<td>47%</td>
</tr>
<tr>
<td><strong>Black, %</strong></td>
<td>49%</td>
<td>47%</td>
</tr>
<tr>
<td><strong>Other, %</strong></td>
<td>8%</td>
<td>6%</td>
</tr>
<tr>
<td><strong>Clinic systolic BP, mm Hg</strong></td>
<td>131 ± 10 (130, 125 - 136)</td>
<td>130 ± 10 (129, 123 - 136)</td>
</tr>
<tr>
<td><strong>Clinic diastolic BP, mm Hg</strong></td>
<td>82 ± 9 (82, 76 - 88)</td>
<td>81 ± 9 (81, 75 - 87)</td>
</tr>
<tr>
<td><strong>24-hour systolic BP, mm Hg</strong></td>
<td>127 ± 10 (127, 120 - 134)</td>
<td>127 ± 9 (127, 121 - 133)</td>
</tr>
<tr>
<td><strong>24-hour diastolic BP, mm Hg</strong></td>
<td>78 ± 8 (78, 73 - 83)</td>
<td>77 ± 8 (77, 72 - 82)</td>
</tr>
<tr>
<td><strong>Daytime systolic BP, mm Hg</strong></td>
<td>130 ± 10 (129, 122 - 137)</td>
<td>130 ± 10 (130, 123 - 136)</td>
</tr>
<tr>
<td><strong>Daytime diastolic BP, mm Hg</strong></td>
<td>81 ± 9 (81, 74 - 86)</td>
<td>80 ± 9 (80, 73 - 85)</td>
</tr>
<tr>
<td><strong>Nighttime systolic BP, mm Hg</strong></td>
<td>121 ± 12 (120, 112 - 128)</td>
<td>121 ± 10 (120, 114 - 127)</td>
</tr>
<tr>
<td><strong>Nighttime diastolic BP, mm Hg</strong></td>
<td>71 ± 10 (70, 64 - 78)</td>
<td>71 ± 9 (70, 64 - 77)</td>
</tr>
<tr>
<td><strong>Site, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boston</td>
<td>184 (70%)</td>
<td>186 (69%)</td>
</tr>
<tr>
<td>Hartford</td>
<td>28 (11%)</td>
<td>31 (12%)</td>
</tr>
<tr>
<td>Minneapolis, Allina Healthcare</td>
<td>36 (14%)</td>
<td>38 (14%)</td>
</tr>
<tr>
<td>Minneapolis, Cultural Wellness Ctr</td>
<td>16 (6%)</td>
<td>15 (6%)</td>
</tr>
<tr>
<td><strong>Season of enrollment, %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>39%</td>
<td>38%</td>
</tr>
<tr>
<td>Spring</td>
<td>23%</td>
<td>27%</td>
</tr>
<tr>
<td>Summer</td>
<td>15%</td>
<td>13%</td>
</tr>
<tr>
<td>Fall</td>
<td>23%</td>
<td>22%</td>
</tr>
</tbody>
</table>

For continuous variables, values are mean ± SD (median, IQR); SD: standard deviation; IQR: interquartile range; BP: blood pressure. Hypertensives are defined on the baseline ABPM status i.e. mean 24-hour systolic or diastolic BP ≥130/80 or mean daytime systolic BP ≥135/85.
Table 2. Results for 24-hour ambulatory blood pressure.

<table>
<thead>
<tr>
<th></th>
<th>High-dose arm</th>
<th>Low-dose arm</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At 6 months</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>127 ± 10 (125, 120 - 133)</td>
<td>126 ± 9 (126, 119 - 132)</td>
<td>0.58</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>77 ± 9 (76, 71 - 83)</td>
<td>76 ± 8 (76, 72 - 81)</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>Change from baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg*</td>
<td>-0.8 ± 8.7 (-1.2, [(-6) - 5])</td>
<td>-1.6 ± 8.8 (-0.4, [(-7) - 4])</td>
<td>0.71</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>-1.2 ± 6.5 (-1.5, [(-5) - 2])</td>
<td>-1.0 ± 6.8 (-0.2, [(-5) - 4])</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Values are mean ± SD (median, IQR); SD: standard deviation; IQR: interquartile range; BP: blood pressure.
*The primary endpoint is the change in 24-hour ambulatory blood pressure between baseline and 6 months. For comparison of change in 24-hour ambulatory blood pressures, p-values are calculated using a non-parametric Wilcoxon rank sum test. For comparison of 6-month blood pressures, p-values are based on analyses of covariance, with race/ethnicity, study site, randomization season, baseline blood pressure, and age by randomization group as covariates.

Table 3. Results for secondary endpoints.

<table>
<thead>
<tr>
<th></th>
<th>High-dose arm (N=188)</th>
<th>Low-dose arm (N=175)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At 6 months</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinic systolic BP, mm Hg</td>
<td>128 ± 12 (127, 120 - 136)</td>
<td>127 ± 11 (125, 120 - 133)</td>
<td>0.88</td>
</tr>
<tr>
<td>Clinic diastolic BP, mm Hg</td>
<td>83 ± 10 (83, 76 - 89)</td>
<td>82 ± 9 (81, 76 - 87)</td>
<td>0.81</td>
</tr>
<tr>
<td>Daytime systolic BP, mm Hg</td>
<td>129 ± 10 (128, 122 - 137)</td>
<td>128 ± 9 (128, 122 - 135)</td>
<td>0.54</td>
</tr>
<tr>
<td>Daytime diastolic BP, mm Hg</td>
<td>80 ± 9 (79, 74 - 85)</td>
<td>79 ± 8 (79, 74 - 84)</td>
<td>0.82</td>
</tr>
<tr>
<td>Nighttime systolic BP, mm Hg</td>
<td>120 ± 12 (118, 112 - 126)</td>
<td>119 ± 10 (118, 113 - 126)</td>
<td>0.33</td>
</tr>
<tr>
<td>Nighttime diastolic BP, mm Hg</td>
<td>70 ± 10 (69, 63 - 76)</td>
<td>70 ± 9 (70, 64 - 75)</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Values are mean ± SD (median, IQR); SD: standard deviation; IQR: interquartile range; BP: blood pressure.
* For clinic blood pressures, p values are based on generalized ordinary least square regression, with the following covariates: baseline blood pressure, study site, days from randomization, randomization arm, and randomization arm by days from randomization interaction. For daytime and nighttime blood pressures, p values are based on ANCOVA, adjusted for race/ethnicity, study site, randomization season, and baseline blood pressure. A race by randomization group term was also included in the ANCOVA model.
Figure Legends:

Figure 1. CONSORT Diagram.

Figure 2. 25-hydroxyvitamin D levels at each study visit, accordingly to treatment group.

Figure 3. Clinic systolic and diastolic blood pressures at each study visit, according to treatment group.

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.

Figure 5. Change in mean 24-hour systolic blood pressure (SBP) between baseline and 6 months, in pre-specified subgroups.
Figure 1

*455 subjects (228 high dose, 227 low dose) had at least one clinic BP and included in analysis of secondary endpoints
Figure 3
Figure 5
Vitamin D Therapy in Individuals with Pre-Hypertension or Hypertension: The DAYLIGHT Trial

Pankaj Arora, Yanna Song, Jeffery Dusek, Gregory Plotnikoff, Marc 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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The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/early/2014/10/30/CIRCULATIONAHA.114.011732

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2014/10/30/CIRCULATIONAHA.114.011732.DC1
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/m²; 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, Bannockburn, 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.
REFERENCES


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

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

For continuous variables, values are mean ± SD (median, IQR); SD: standard deviation; IQR: interquartile range; BP: blood pressure.
Supplementary Figure 1