Effect of Cholecalciferol Supplementation During Winter Months in Patients With Hypertension: A Randomized, Placebo-Controlled Trial

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BACKGROUND
Low 25-hydroxy-vitamin D (25(OH)D) levels are inversely related to blood pressure (BP) and have been associated with incident hypertension. In people living at northern latitudes diminished cholecalciferol synthesis in the winter increases the risk of vitamin D deficiency. We wanted to test the hypothesis that daily cholecalciferol supplementation in the winter lowers BP in patients with hypertension.

METHODS
We investigated the effect of 75 µg (3,000 IU) cholecalciferol per day in a randomized, placebo-controlled, double-blind study in 130 hypertensive patients residing in Denmark (56° N). Ambulatory BP (24-h BP) and arterial stiffness were measured before and after 20 weeks of treatment, that took place between October and March.

RESULTS
A total of 112 patients (mean age 61 ± 10) with a baseline p-25(OH)D of 23 ± 10 ng/ml completed the study. Compared with placebo, a nonsignificant 3/1 mm Hg ($P = 0.26/0.18$) reduction was found in 24-h BP. In patients with vitamin D insufficiency (<32 ng/ml) at baseline ($n = 92$), 24-h BP decreased by 4/3 mm Hg ($P = 0.05/0.01$). Central BP (CBP) estimated by applanation tonometry and calibrated with a standardized office BP was reduced by 7/2 mm Hg ($P = 0.007/0.15$) vs. placebo. No differences in carotid-femoral pulse wave velocity (PWV) or central augmentation index (AIx) were found between treatment arms.

CONCLUSIONS
Cholecalciferol supplementation, by a dose that effectively increased vitamin D levels, did not reduce 24-h BP, although central systolic BP decreased significantly. In a post-hoc subgroup analysis of 92 subjects with baseline p-25(OH)D levels <32 ng/ml, significant decreases in 24-h systolic and diastolic BP occurred during cholecalciferol supplementation.

Keywords: *arterial stiffness; blood pressure; cholecalciferol; hypertension; winter*

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Vitamin D has its primary functions in the musculoskeletal system and its role in preventing osteomalacia and rickets is well characterized.[1] In recent years, vitamin D deficiency has been linked to diseases in many other organ systems including the cardiovascular system,[2,3] and it has been shown that the ubiquitously present vitamin D receptor regulates a large number of genes not involved in calcium metabolism.[4] Moreover, many tissues in the human body including vascular endothelial cells and cardiomyocytes express 1α-hydroxylase,[5–7] suggesting that vitamin D also functions in para- and autocrine ways.

Solar ultraviolet B (UVB) radiation is our most important source of vitamin D,[8] and accordingly plasma levels of 25--hydroxy-vitamin D (25(OH)D) exhibit seasonal variations.[9,10] In populations studies, p-25(OH)D is inversely correlated with blood pressure (BP), and vitamin D deficiency has been linked to both prevalence of hypertension and risk of incident hypertension.[11–13] However, poor vitamin D status is also associated with other cardiovascular risk factors such as obesity, smoking, sedentary lifestyle, and unhealthy diet,[10,14] therefore raising the question whether true causal relations exist.[15] Results from clinical trials investigating the effect of vitamin D on BP have been inconsistent, and neither of two recent meta-analyses found strong evidence to support a substantial effect of vitamin D.[16,17]

The discrepancy in findings from clinical trials to date can be related to heterogeneity in study populations, low vitamin D dosages, short duration of treatment, nonhypertensive populations, and confounding solar UVB radiation.[18] Thus, randomized controlled trials establishing the effect of vitamin D supplementation on BP are still mandated. In this study, we tested the hypothesis that daily cholecalciferol supplementation during winter months lowers BP in patients with hypertension.

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METHODS

Study population. In the late summer and fall of 2010, patients with hypertension were recruited by advertisements in local newspapers and through pamphlets placed at general practitioners' offices in the municipality of Holstebro, Denmark. Inclusion criteria were arterial hypertension, Caucasian race, and residence in Denmark (56° N). Exclusion criteria included 24-h ambulatory BP (24-h BP) >150 mm Hg systolic and/or 95 mm Hg diastolic, malignant disease, atrial flutter or fibrillation, hypercalcemia, pregnancy or nursing, alcohol abuse (>24 g of alcohol per day for women and >36 for men), regular use of nonsteroidal anti-inflammatory drugs or glucocorticoids, daily vitamin D intake exceeding 10 µg of chole- or ergocalciferol, tanning bed usage, and changes in antihypertensive medication during trial period. Recruitment and follow-up took place between 4 October 2010 and 31 March 2011.

Ethics. The study was approved by the Regional Committee on Biomedical Research Ethics (j. no. M-20100120), and carried out in accordance with the Declaration of Helsinki. Written, informed consent was obtained from each subject. The study was implemented without any changes to the original protocol. http://www.ClinicalTrials.gov no.: NCT01166165.

Randomization. Participants were allocated to treatment via permutated block randomization conducted at http://www.randomization.com by the hospital pharmacy. Cases 1–120 were randomized in one block and 121–130 in five blocks of two. The hospital pharmacy generated the randomization sequence and labeled the bottles. After baseline examination at the clinic but before ambulatory BP monitoring, participants sequentially received a numbered bottle containing either cholecalciferol or matching placebo tablets. Cholecalciferol and placebo tablets were identical in size, color, shape, consistency, taste, and ingredients except from cholecalciferol. The randomization code was kept in a sealed envelope until after the last visit of the last participant. Investigators, participants, and other study personnel were blinded to treatment assignment for the duration of the study.

Effect variable. The primary effect variable was 24-h systolic BP. Secondary effect variables were 24-h diastolic BP and heart rate, central BP (CBP), central augmentation index (AIx), carotid-femoral pulse wave velocity (PWV), urinary calcium-creatinine ratio, and plasma levels of renin (PRC), angiotensin II (Ang II), aldosterone (ALDO), brain natriuretic peptide, 25(OH)D, intact parathyroid hormone (PTH), ionized calcium (Ca^{++}), phosphate, and fibroblast growth factor 23 (FGF23).

Experimental procedure. During the 20-week randomized, placebo-controlled, double-blind study, participants were allocated to either three tablets of 25 µg cholecalciferol daily or matching placebo. Participants taking multivitamins were allowed to take a maximum of 10 µg of ergo- or cholecalciferol daily. At the day of baseline and follow-up examinations participants took their usual medication, and were not allowed any alcoholic or caffeinated beverages. At arrival at the research facility, a spot urine sample was collected, and supine position was assumed in a quiet temperature controlled room (temperature range 21–24 °C). After 30 min, applanation tonometry was performed, immediately followed by venous blood sampling and mounting of 24-h BP equipment. Between baseline and follow-up, blood samples were obtained every 5 weeks for safety assessment of plasma concentrations Ca^{++}, phosphate, and p-25(OH)D.

BP measurements. Twenty-four hour BP was measured using Kivex TM-2430 (Kivex, Hoersholm, Denmark). Measurements were taken every 15 min during daytime and every 30 min overnight. PWV and radial pulse wave analysis were obtained by a trained technician using applanation tonometry (SphygmoCor CPV system; Atcor Medical, Sydney, Australia). Only duplicate recordings that met the minimum quality requirements were included in the final analysis. The same technician recorded brachial office BP for calibration of the SphygmoCor system by a semiautomatic, oscillometric device (Omron 705IT; Omron, Tokyo, Japan). If the difference within a duplicate BP recording exceeded 7 mm Hg, the BP measurement was repeated.

Laboratory analyses. Routine blood and urine samples obtained at baseline and every 5 weeks throughout the study were immediately assayed at the Department of Clinical Biochemistry. Commercial chemiluminescence immunoassays were used to analyze plasma concentrations of 25(OH)D2+D3 (Liaison; DiaSorin, Saluggia, Italy) and brain natriuretic peptide (Centaur, Bayer, Germany). Remaining blood samples were taken on ice and centrifuged for 15 min at 3,500 rpm and kept frozen at −80 °C (renin, ALDO, and FGF23) and −20 °C (Ang II) until assayed. Plasma renin was determined using an immunoradiometric assay from CIS Bio International, Gif-Sur-Yvette Cedex, France. Minimal detection level was 1 pg/ml. The coefficients of variation were 0.9–3.6% (intra-assay) and 3.7–5.0% (inter-assay) in the range of 4–263 pg/ml. Ang II was extracted from plasma with C_{18} Sep-Pak (Water associates, Milford, MA), and subsequently determined by radioimmunoassay as previously described.[19] The antibody was obtained from the Department of Clinical Physiology, Glostrup Hospital, Glostrup, Denmark. Minimal detection level was 1.5 pmol/l. Minimal detection level was 1.5 pg/ml. The coefficients of variation were 12% (inter-assay) and 8% (intra-assay). ALDO was determined by radioimmunoassay using a kit from Demeditec Diagnostics, Kiel, Germany. Minimal detection level was 25 pmol/l. The coefficients of variation were 9.0% (inter-assay) and 8.5% (intra-assay). Intact plasma levels of FGF23 were determined by a sandwich ELISA (Immutopics, San Clemente, CA). The coefficients of variation were 6% (inter-assay) and 4% (intra-assay).

Study drug. Cholecalciferol (25 µg) and placebo tablets were obtained from Ferrosan A/S, Soeborg, Denmark.

Statistical methods. Sample size was calculated using 24-h systolic BP as primary endpoint. With a significance level of

5% and a 90% power to detect a 5 mm Hg difference in BP (s.d. 8 mm Hg), 54 patients were needed in each of the two groups. Baseline characteristics between groups were compared by an independent *t*-test for continuous variables and χ^2-test for categorical variables. Differences between changes in the two treatment groups were compared using an independent *t*-test if effect variables were parametric, and the Mann–Whitney U-test for nonparametric variables. A general linear model with repeated measures was applied to assess changes in p-25(OH)D during the course of the trial. Parametric data are presented as means ± s.d., nonparametric data as medians with 25th and 75th percentiles. Statistical significance was defined as $P < 0.05$. Statistical analyses were performed using PASW version 18.0.0 (SPSS, Chicago, IL).

RESULTS

Demographics

A total of 136 patients were screened and 130 were found eligible for entering the study (**Figure 1**). Eighteen patients were excluded due to withdrawal of consent (6), 24-h systolic BP >150 mm Hg (5), inability to complete 24-h BP measurement (2), changes in antihypertensive medication (2), ibuprofen treatment (1), cancer (1), and major surgery close to follow-up (1). Thus, 112 patients completed the trial. Mean compliance by pill count was 99% in both groups. Baseline characteristics were similar in the two groups with the exception of plasma concentrations of ionized calcium (p-Ca^{++}) and alkaline phosphatase (p-ALP) that were higher in the placebo group (**Table 1**). At baseline, p-25(OH)D was <32 ng/ml in 92 patients (82%), and <20 ng/ml in 47 patients (42%).

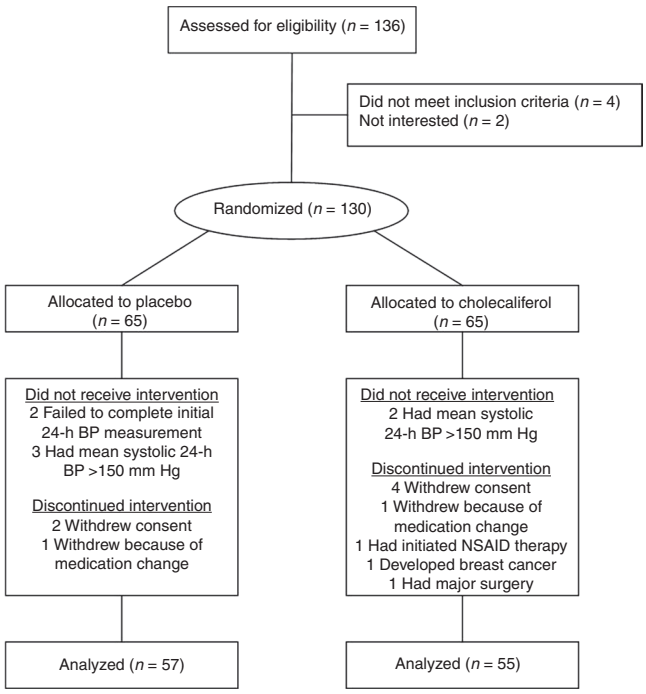

Figure 1 | Flow chart. BP, blood pressure; NSAID, nonsteroidal anti-inflammatory drugs.

Six patients (three in each group) developed a truncal, pruritic elevated rash during treatment. None of the patients discontinued treatment. No other side effects were reported.

During the course of the trial, nine patients spent time (maximum 2 weeks) at latitudes or altitudes compatible with cutaneous vitamin D synthesis. Of these, patients allocated to cholecalciferol ($n = 4$) had the same mean increase in p-25(OH)D as those who were not exposed to UVB radiation. In patients allocated to placebo ($n = 5$), p-25(OH)D decreased by 1 ng/ml (interquartile range: −1; 4) in travellers, as opposed to non-travellers who had a 3 ng/ml (interquartile range: −6; −1) decrease in p-25(OH)D ($P = 0.053$, Mann–Whitney U-test).

Table 1 | Baseline characteristics according to randomization

	Placebo ($n = 57$)	Cholecalciferol ($n = 55$)	*P* value
Age (years)	61 ± 9	60 ± 12	0.78
Male sex, *n* (%)	18 (32)	17 (30)	0.94
Smokers, *n* (%)			
Current	5 (9)	4 (7)	0.77
Former	17 (30)	11 (20)	0.23
Never	35 (61)	40 (73)	0.20
Type 2 diabetes, *n* (%)	5 (9)	4 (7)	0.75
Body mass index (kg/m^2)	28.3 ± 3.7	27.7 ± 4.2	0.41
24-h BP (mm Hg)	131/77 ± 9/6	132/77 ± 10/6	0.74/0.70
Medication, *n* (%)			
ACEi/ARBs	48 (84)	47 (84)	0.85
Calcium channel blockers	22 (39)	20 (36)	0.81
β-Blockers	12 (21)	19 (34)	0.11
Loop diuretics	3 (5)	6 (11)	0.27
Thiazide diuretics	37 (65)	28 (50)	0.13
Potassium-sparing diuretics	2 (4)	1 (2)	0.58
Statins	19 (33)	17 (30)	0.78
Bisphosphonates	0 (0)	2 (4)	0.15
Multivitamins[a]	18 (32)	14 (25)	0.47
Routine biochemistry			
p-25(OH)D, ng/ml	23 ± 12	23 ± 9	0.74
p-iPTH, pg/ml	41.4 ± 9.8	42.7 ± 15.3	0.58
p-Ca^{++}, mg/dl	5.04 ± 0.16	4.92 ± 0.12	0.003
p-Phosphate, mg/dl	3.10 ± 0.50	3.07 ± 0.46	0.70
p-Albumin, g/dl	4.2 ± 0.3	4.2 ± 0.2	0.90
p-ALP, U/l	70 ± 21	61 ± 16	0.01
p-CRP, mg/l	2.8 ± 2.5	2.6 ± 3.3	0.74
p-Creatinine, mg/dl	0.83 ± 0.21	0.81 ± 0.15	0.74

Values are mean ± s.d. To convert 25(OH)D from ng/ml to nmol/l multiply by 2.496; iPTH from pg/ml to pmol/l multiply by 0.1061; Ca^{++} from mg/dl to nmol/l multiply by 0.25; phosphate from mg/dl to mmol/l multiply by 0.323; creatinine from mg/l to μmol/l multiply by 88.4.
25(OH) D, 25-hydroxy-vitamin D; ACEi, angiotensin-converting enzyme inhibitor; ALP, alkaline phosphatase; ARBs, angiotensin receptor blockers; BP, blood pressure; bpm, beats per minute; Ca^{++}, ionized calcium; CRP, C-reactive protein; iPTH, intact parathyroid hormone.
[a]≤10 μg ergo- or cholecalciferol per day.

Calcium metabolism

Plasma levels of PTH, phosphate, and Ca^{++} levels remained within normal ranges throughout the study (Tables 2 and 3). As depicted in Figure 2, the p-25(OH)D level increased by 21 ± 10 ng/ml ($P < 0.001$) in the cholecalciferol group and decreased by 3 ± 6 ng/ml ($P < 0.001$) in the placebo group. Cholecalciferol increased p-Ca^{++} (0.06 ± 0.11 mg/dl, $P < 0.05$), and suppressed p-PTH (−9.2 ± 8.8 pg/ml, $P < 0.001$), vs. placebo. Changes in p-25(OH)D correlated negatively with changes in p-PTH (Pearson: $r = -0.48$, $P < 0.001$, $n = 112$), and less pronounced, there was a weak positive correlation between changes in p-PTH and changes in 24-h systolic BP (Pearson: $r = 0.28$, $P = 0.04$, $n = 112$). Cholecalciferol did not cause any significant changes in p-phosphate ($P = 0.42$), p-FGF23 ($P = 0.23$) or urinary calcium/creatinine ratio ($P = 0.90$), vs. placebo.

Table 2 | Effect of cholecalciferol on calcium metabolism and vasoactive hormones

	Baseline	20 weeks	*P* value
p-iPTH (pg/ml)			
Placebo ($n = 57$)	41.4 ± 9.8	42.1 ± 10.2	<0.001
Cholecalciferol ($n = 55$)	42.7 ± 15.3	34.3 ± 10.9*	
p-Ca^{++} (mg/dl)			
Placebo ($n = 57$)	5.04 ± 0.16	4.99 ± 0.18	0.009
Cholecalciferol ($n = 55$)	4.94 ± 0.12	4.96 ± 0.13	
p-Phosphate (mg/dl)			
Placebo ($n = 57$)	3.10 ± 0.50	3.28 ± 0.40*	0.42
Cholecalciferol ($n = 55$)	3.07 ± 0.46	3.22 ± 0.59*	
p-Potassium (mEq/l)			
Placebo ($n = 57$)	3.9 ± 0.3	3.9 ± 0.3	0.63
Cholecalciferol ($n = 55$)	3.9 ± 0.4	3.9 ± 0.3	
p-FGF23 (pg/ml)			
Placebo ($n = 57$)	11.1 (8.6; 13.0)	10.3 (8.8; 12.8)	0.23
Cholecalciferol ($n = 55$)	9.1 (8.0; 11.9)	9.2 (7.9; 11.7)	
u-Calcium/creatinine ratio			
Placebo ($n = 57$)	0.22 (0.14; 0.34)	0.19 (0.13; 0.39)	0.90
Cholecalciferol ($n = 55$)	0.31 (0.20; 0.46)	0.28 (0.17; 0.47)	
Brain natriuretic peptide (pg/ml)			
Placebo ($n = 57$)	17.3 (8.3; 39.8)	17.3 (12.5; 39.1)	0.12
Cholecalciferol ($n = 55$)	21.5 (13.8; 38.1)	20.8 (15.2; 30.4)	
Plasma renin concentration (pg/ml)			
Placebo ($n = 57$)	20.2 (10.3; 51.6)	11.6 (7.2; 27.7)*	0.07
Cholecalciferol ($n = 55$)	18.3 (6.0; 39.2)	18.3 (4.4; 65.2)	
Angiotensin II (pg/ml)			
Placebo ($n = 57$)	11 (6; 17)	7 (5; 16)*	0.49
Cholecalciferol ($n = 55$)	7 (4; 20)	6 (4; 15)	
Aldosterone (ng/dl)			
Placebo ($n = 57$)	4.9 (3.7; 7.7)	4.8 (3.8; 5.8)	0.23
Cholecalciferol ($n = 55$)	4.7 (3.2; 6.5)	4.0 (3.1; 6.5)	

Values are mean ± s.d. or medians with 25 and 75 percentiles in brackets. To convert iPTH from pg/ml to pmol/l multiply by 0.1061; Ca^{++} from mg/dl to nmol/l multiply by 0.25; phosphate from mg/dl to mmol/l multiply by 0.323; B-type natriuretic peptide from pg/ml to pmol/l multiply by 0.289; aldosterone from ng/dl to pmol/l multiply by 27.7. *P* values represent the probability of a difference between treatment groups.
Ca^{++}, ionized calcium; FGF23, fibroblast growth factor 23; iPTH, intact parathyroid hormone.
*$P < 0.05$ vs. baseline.

BP and arterial stiffness

Cholecalciferol caused a minor nonsignificant reduction in 24-h BP of 3/1 mm Hg ($P = 0.26/0.18$) vs. placebo (Tables 3 and 4). In patients with p-25(OH)D <32 ng/ml at baseline (placebo: $n = 46$; cholecalciferol: $n = 46$), 24-h BP was reduced by 4/3 mm Hg ($P = 0.05/0.01$) (Figure 3). The modest reduction was similar in day- and nighttime measurements, and cholecalciferol neither affected dipper status ($P = 0.27$) nor night-to-day systolic BP ratio ($P = 0.98$).

CBP and measures of arterial stiffness are documented in Tables 3 and 4. Radial pulse wave analysis failed in five patients due to anatomy or prior surgery in that region. In one of these patients pulse wave velocity could not be measured either. Compared with placebo, cholecalciferol reduced CBP by 7/2 mm Hg ($P = 0.007/0.15$). A similar 6/2 mm Hg reduction

Table 3 | Changes from baseline in predefined primary and secondary outcomes after 20 weeks of cholecalciferol supplementation

	Placebo	Cholecalciferol	*P* value
24-h SBP (mm Hg)	0.4 (−1.8 to 2.7)	−1.5 (−4.0 to 1.1)	0.26
24-h DBP (mm Hg)	0.2 (−1.1 to 1.6)	−1.1 (−2.5 to 0.3)	0.18
24-h heart rate (bpm)	−0.6 (−1.8 to 0.6)	0.2 (−1.2 to 1.5)	0.37
Central SBP (mm Hg)[a]	1.8 (−2.1 to 5.6)	−5.0 (−8.2 to −1.9)	0.007
Central DBP (mm Hg)[a]	−0.9 (−2.6 to 0.8)	−2.6 (−4.2 to −0.9)	0.15
Central augmentation index (%)[a]	0.1 (−1.3 to 1.6)	−0.8 (−2.2 to 0.6)	0.37
Pulse wave velocity (m/s)[b]	0.3 (0.0 to 0.7)	0.4 (0.2 to 0.7)	0.66
p-25(OH)D (ng/ml)	−3 (−5 to −2)	21 (19 to 24)	<0.001
p-iPTH	0.8 (−1.0 to 2.6)	−8.4 (−11.3 to −5.5)	<0.001
p-Ca^{++} (mg/dl)	−0.03 (−0.06 to 0.00)	0.03 (0.00 to 0.06)	0.009
p-Phosphate (mg/dl)	0.18 (0.07 to 0.28)	0.16 (0.05 to 0.27)	0.78
p-FGF23 (pg/ml)*	0.0 (−4.0; 1.5)	0.2 (−1.6; 2.0)	0.23
u-Calcium/creatinine ratio*	0.0 (−0.2; 0.2)	0.0 (−0.3; 0.2)	0.90
PRC (pg/ml)*	−3.7 (−72.8; 12.2)	−0.3 (−47.2; 58.7)	0.07
p-Angiotensin II (pg/ml)*	−2 (−18; 5)	−1 (−14; 6)	0.49
p-Aldosterone (ng/dl)*	−0.3 (−4.5; 1.4)	0.0 (−2.9; 3.1)	0.23
p-BNP (pg/ml)*	0.3 (−19.4; 29.0)	−1.4 (−18.4; 12.5)	0.12

Values are means with 95% confidence intervals in brackets, except variables marked with * (asterisks) which are medians with 10 and 90 percentiles in brackets. *P* values in right column represent the probability of a difference between treatment groups.
25(OH)D, 25-hydroxy-vitamin D; BNP, brain natriuretic peptide; bpm, beats per minute; Ca^{++}, ionized calcium; DBP, diastolic blood pressure; FGF23, fibroblast growth factor 23; iPTH, intact parathyroid hormone; PRC, plasma levels of rennin; SBP, systolic blood pressure.
[a]Measurements available in 107 patients. [b]Measurements available in 111 patients.

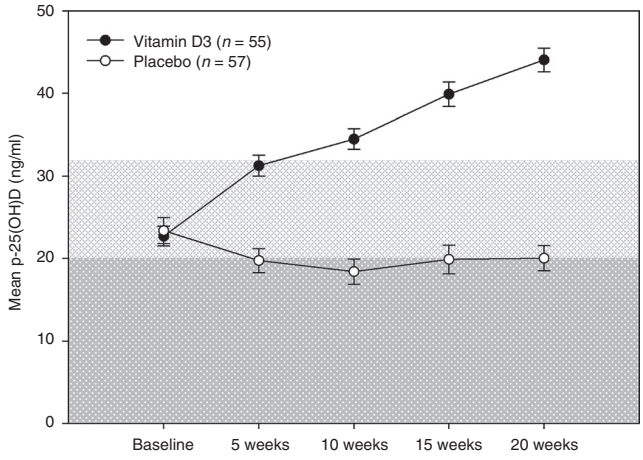

Figure 2 | Mean plasma concentrations of 25-hydroxy-vitamin D (p-25(OH)D) with s.e. From "5 weeks" onward p-25(OH)D was significantly improved ($P < 0.001$), whereas p-25(OH)D dropped significantly in the placebo group ($P < 0.001$). Light gray area represents vitamin D insufficiency (p-25(OH)D <32 ng/ml). Dark gray area represents vitamin D deficiency (p25(OH)D <20 ng/ml).

(P = 0.02/0.18) was observed in brachial BP obtained immediately before applanation tonometry. No changes between groups were observed in AIx (P = 0.37) or PWV (P = 0.66). However, from fall to late winter, PWV tended to increase in both the cholecalciferol group (0.45 ± 0.11 m/s, P = 0.004) and the placebo group (0.34 ± 0.13 m/s, P = 0.06).

Vasoactive hormones

Changes in brain natriuretic peptide, PRC, Ang II or ALDO are documented in **Tables 2** and **3**. Median changes between baseline and follow-up in PRC and Ang II did not differ significantly between groups (P = 0.07 and P = 0.49, respectively) although the largest suppression of these hormones seemed to occur in the placebo group.

DISCUSSION

Daily cholecalciferol supplementation during winter months did not lower 24-h BP significantly in patients with hypertension. However, a marked and highly significant reduction was observed in CBP, which was a secondary endpoint. While the optimal vitamin D level for cardiovascular health remains to be established, vitamin D sufficiency as it relates to optimal levels of biomarkers for calcium metabolism has been defined as p-25(OH)D≥32 ng/ml.[20,21] Below this threshold PTH will start to rise.[22,23] In our post-hoc subanalysis, we excluded patients who were vitamin D sufficient at baseline and found that treatment with cholecalciferol significantly reduced both systolic and diastolic 24-h BP.

In individuals with vitamin D deficiency (p-25(OH)D <20 ng/ml), reductions in systolic office BP have been reported after vitamin D supplementation in both type 2 diabetics and nondiabetics,[24–26] and UVB irradiation significantly lowered both systolic and diastolic ambulatory BP.[27] Brachial office BP was not a predefined endpoint in our study, but was obtained for the purpose of calibrating the SphygmoCor system. Our data showed that vitamin D had a pronounced effect on office

Table 4 | Effect of cholecalciferol on blood pressure and measures of arterial stiffness

	Baseline	20 weeks	*P* value
24-h SBP (mm Hg)			
Placebo (*n* = 57)	131 ± 9	132 ± 11	0.26
Cholecalciferol (*n* = 55)	132 ± 10	130 ± 11	
24-h DBP (mm Hg)			
Placebo (*n* = 57)	77 ± 6	77 ± 7	0.18
Cholecalciferol (*n* = 55)	77 ± 6	76 ± 7	
24-h heart rate (bpm)			
Placebo (*n* = 57)	72 ± 10	72 ± 9	0.37
Cholecalciferol (*n* = 55)	67 ± 8	67 ± 8	
24-h daytime SBP (mm Hg)			
Placebo (*n* = 57)	136 ± 9	136 ± 11	0.28
Cholecalciferol (*n* = 55)	137 ± 10	135 ± 12	
24-h daytime DBP (mm Hg)			
Placebo (*n* = 57)	79 ± 7	80 ± 8	0.19
Cholecalciferol (*n* = 55)	80 ± 7	79 ± 7	
24-h daytime heart rate (bpm)			
Placebo (*n* = 57)	75 ± 10	75 ± 10	0.29
Cholecalciferol (*n* = 55)	70 ± 8	70 ± 9	
24-h nighttime SBP (mm Hg)			
Placebo (*n* = 57)	117 ± 10	117 ± 12	0.30
Cholecalciferol (*n* = 55)	117 ± 11	115 ± 12	
24-h nighttime DBP (mm Hg)			
Placebo (*n* = 57)	68 ± 7	68 ± 7	0.22
Cholecalciferol (*n* = 55)	68 ± 7	67 ± 7	
24-h nighttime heart rate (bpm)			
Placebo (*n* = 57)	63 ± 9	63 ± 10	0.65
Cholecalciferol (*n* = 55)	60 ± 7	59 ± 8	
Pulse wave velocity (m/s)[a]			
Placebo (*n* = 57)	8.7 ± 2.1	9.0 ± 2.4	0.66
D3 (*n* = 54)	8.5 ± 2.3	9.0 ± 2.5*	
Augmentation index (%)[b]			
Placebo (*n* = 55)	26 ± 9	26 ± 8	0.37
D3 (*n* = 52)	26 ± 7	25 ± 9	
Central SBP (mm Hg)[b]			
Placebo (*n* = 55)	132 ± 13	133 ± 15	0.007
D3 (*n* = 52)	135 ± 16	130 ± 18*	
Central DBP (mm Hg)[b]			
Placebo (*n* = 55)	82 ± 8	81 ± 8	0.15
D3 (*n* = 52)	83 ± 8	80 ± 9*	
Office SBP (mm Hg)[b]			
Placebo (*n* = 55)	142 ± 13	143 ± 15	0.02
D3 (*n* = 52)	144 ± 16	139 ± 18*	
Office DBP (mm Hg)[b]			
Placebo (*n* = 55)	81 ± 8	80 ± 7	0.18
D3 (*n* = 52)	81 ± 11	79 ± 9*	

Values are means ± s.d. *P* values in right column represent the probability of a difference between treatment groups.
bpm, beats per minute; DBP, diastolic blood pressure; SBP, systolic blood pressure.
[a]Measurements available in 111 patients. [b]Measurements available in 107 patients.
*$P < 0.05$ vs. baseline.

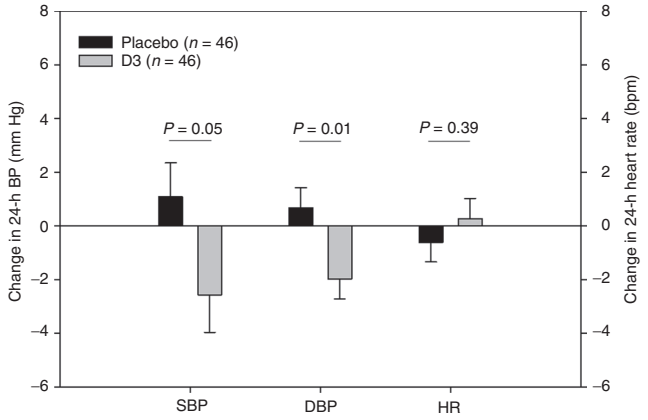

Figure 3 | Ambulatory blood pressure (BP) and heart rate in patients with plasma concentrations of 25–hydroxy–vitamin D <80 nmol/l at baseline (n = 92). Mean values and s.e.m. after treatment with cholecalciferol and placebo. bpm, beats per minute; DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure.

BP, which may explain the reduction in CBP. Twenty-four hour BP is considered superior to office BP because of increased reproducibility.[28] Standard deviations in 24-h BP and overlapping confidence intervals between changes in systolic office BP and systolic 24-h BP suggest that we might have found a significant effect on 24-h BP if more patients had been included in the study. The differences in the findings concerning office and ambulatory measurements may be chance findings caused by slightly insufficient power.

There are several possible mechanisms by which vitamin D may lower BP, including effects on the renin–angiotensin system (RAS), PTH as well as direct modulatory effects on vascular smooth muscle cells. First, in animal studies $1,25(OH)_2D$ downregulates renin expression independently from PTH and calcium levels,[29] and renin expression is highly elevated in vitamin D receptor null mice, which leads to systemic hypertension, cardiac hypertrophy, and heart failure.[30–32] Although inverse correlations between p-25(OH)D and plasma renin activity have been documented in both normotensive and hypertensive populations,[33–35] prospective human clinical trials have not confirmed this relationship satisfactorily. Conversely, Sugden *et al.* reported a highly significant decrease in systolic BP 8 weeks after a single oral dose of 2.5 mg ergocalciferol without significant effects on PRC.[26] The results of a descriptive study of 17 children and young adults with hereditary vitamin D resistant rickets were also remarkable, as none of the patients had hypertension, left ventricular hypertrophy or plasma renin activity elevation.[36] Thus, the role of vitamin D in the complex regulation of renin expression has not been fully established in humans. In our study, cholecalciferol treatment did not alter plasma levels of RAS components significantly. On the contrary, PRC and Ang II tended to decrease. Given that most patients were on an angiotensin-converting enzyme inhibitor or Ang II receptor blocker, conclusions regarding changes in the RAS should be made with a certain reservation.

Second, the PTH receptor has been identified in vascular endothelium and smooth muscle cells,[37] which suggests that PTH may have direct regulatory effects on the vessel wall.[38] Epidemiologic studies have shown independent associations between PTH and BP,[39,40] and most patients with primary hyperparathyroidism are hypertensive.[41] In addition, low calcium levels have been shown to contribute to hypertension.[42] In experimental studies, BP increases during PTH infusion,[43] and PTH was a significant predictor of BP in elderly patients with hypertension.[44] Cross-sectional data from the US National Health and Nutrition Examination Surveys suggest that the association between vitamin D and BP may in fact be mediated by PTH.[45] In the present study, cholecalciferol significantly suppressed PTH and increased Ca^{++} suggesting that at least part of the BP lowering effect of cholecalciferol may be PTH-mediated. Although there was a highly significant negative correlation between changes in 25(OH)D and PTH, only a weak positive correlation was observed between changes in PTH and systolic 24-h BP. At baseline, p-Ca^{++} and p-ALP were significantly higher in the placebo group. Although these findings were seemingly unrelated to PTH and vitamin D status, the subsequent increase in p-Ca^{++} in cholecalciferol-treated patients should therefore be interpreted with caution. Neither plasma levels of phosphate nor FGF23 differed between treatment groups, which are not unexpected in a population with preserved kidney function.

Third, endothelial cells and cardiomyocytes express 1α-hydroxylase, and $1,25(OH)_2D$ modulates the effects of inflammatory cytokines on the vasculature.[5] In rat cell cultures, $1,25(OH)_2D$ caused a pronounced inhibition on vascular smooth cell growth.[46] In vitamin D deficient patients with type 2 diabetes, ergocalciferol improved endothelial function independently from changes in BP.[25] In our study neither PWV nor AIx were reduced significantly, indicating that arterial stiffness was unaffected by cholecalciferol. In both groups, PWV tended to increase from fall to late winter, which is consistent with previous observations that PWV is highest in the winter among hypertensive patients.[47] These findings do not preclude; however, that vitamin D may have a direct effect on vascular resistance, and the study was not sufficiently powered to detect drug-related changes in PWV and AIx.

The study was conducted between October and March in Denmark (56° N) when cutaneous cholecalciferol synthesis is negligible.[9] Vitamin D deficiency was slightly less prevalent compared to what has previously been described at these latitudes.[10] We attribute this to the fact that baseline examinations took place in late fall where plasma levels of vitamin D reach yearly peak values.[10] Although a few patients spent vacation time at southern latitudes during the course of the trial, this did not result in a statistically significant difference in vitamin D status between travellers and non-travellers. However, travellers receiving placebo did tend to have slightly higher vitamin D levels at follow-up. A cholecalciferol dose of 75 μg/day was highly effective in raising vitamin levels without causing hypercalcemia. Similarly findings were reported by Heaney *et al.* who employed cholecalciferol doses up to 275 μg,[48] indicating that the upper tolerable vitamin D input level is much higher than previously assumed. Considering the 2-month whole-body

half-life of cholecalciferol,[49] steady state conditions would most likely require more than 20 weeks of daily supplementation to take place. Thus, future studies should aim at securing a high p-25(OH)D plateau for an extended period of time.

This study had several limitations. Most importantly, the inclusion of patients with sufficient vitamin D levels most likely diminished the effect of vitamin D supplementation. Excluding patients with sufficient vitamin D levels subsequently caused the post-hoc analysis to be underpowered. Furthermore, we tested the effect of cholecalciferol in patients who were already receiving antihypertensive treatment. This constitutes a source of error as it pertains to RAS components in particular, but might also influence vitamin D's effect on BP regulation in other ways. We sought to compensate for this by securing that no changes were made in prescription or nonprescription medication during the study. Due to ethical reasons, we chose a rather conservative exclusion criterion regarding BP. This may have caused us to miss an effect in patients with highly elevated BP, who may have benefited more from vitamin D supplementation than patients with well-treated hypertension. Also, we did not obtain information regarding white coat hypertension. Thus, some patients with white coat hypertension may have been included in the trial. We did not employ any dietary standardizations or recordings of dietary habits. However, the poor vitamin D content in a typical western diet is less likely to have confounded the results.[10] Blinding was complete for participants, investigators, and laboratory technicians for the duration of the trial. Major strengths of the study were its design as a randomized, placebo-controlled trial, the use of longitudinal measurements of p-25(OH)D, the employment of a high dose of vitamin D, and a very high compliance rate.

In conclusion, we found that 24-h systolic BP, which was the primary endpoint of this study, was not significantly affected by cholecalciferol supplementation during winter months in hypertensive Caucasians residing at 56° N. However, patients with vitamin D levels ≤32 ng/ml at baseline had a reduction of borderline significance in both systolic and diastolic 24-h BP. In addition, we found a highly significant effect of cholecalciferol on central systolic BP. Vitamin D's effect on BP did not seem to be mediated by changes in the RAS, but rather through an impact on PTH and calcium balance. Larger randomized controlled trials in vitamin D deficient populations are needed to further elucidate the effects of cholecalciferol on the cardiovascular system.

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1. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; 357:266–281.
2. Leu M, Giovannucci E. Vitamin D: epidemiology of cardiovascular risks and events. *Best Pract Res Clin Endocrinol Metab* 2011; 25:633–646.
3. Zittermann A. Vitamin D and disease prevention with special reference to cardiovascular disease. *Prog Biophys Mol Biol* 2006; 92:39–48.
4. Haussler MR, Whitfield GK, Haussler CA, Hsieh JC, Thompson PD, Selznick SH, Dominguez CE, Jurutka PW. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J Bone Miner Res* 1998; 13:325–349.
5. Zehnder D, Bland R, Chana RS, Wheeler DC, Howie AJ, Williams MC, Stewart PM, Hewison M. Synthesis of 1,25-dihydroxyvitamin D(3) by human endothelial cells is regulated by inflammatory cytokines: a novel autocrine determinant of vascular cell adhesion. *J Am Soc Nephrol* 2002; 13:621–629.
6. Mitsuhashi T, Morris RC Jr, Ives HE. 1,25-dihydroxyvitamin D3 modulates growth of vascular smooth muscle cells. *J Clin Invest* 1991; 87:1889–1895.
7. Merke J, Milde P, Lewicka S, Hügel U, Klaus G, Mangelsdorf DJ, Haussler MR, Rauterberg 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 and human dermal capillaries. *J Clin Invest* 1989; 83:1903–1915.
8. DeLuca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* 2004; 80:1689S–1696S.
9. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Metab* 1988; 67:373–378.
10. Thuesen B, Husemoen L, Fenger M, Jakobsen J, Schwarz P, Toft U, Ovesen L, Jørgensen T, Linneberg A. Determinants of vitamin D status in a general population of Danish adults. *Bone* 2012; 50:605–610.
11. Burgaz A, Orsini N, Larsson SC, Wolk A. Blood 25-hydroxyvitamin D concentration and hypertension: a meta-analysis. *J Hypertens* 2011; 29:636–645.
12. Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC, Curhan GC. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension* 2007; 49:1063–1069.
13. Judd SE, Nanes MS, Ziegler TR, Wilson PW, Tangpricha V. Optimal vitamin D status attenuates the age-associated increase in systolic blood pressure in white Americans: results from the third National Health and Nutrition Examination Survey. *Am J Clin Nutr* 2008; 87:136–141.
14. Borges MC, Martini LA, Rogero MM. Current perspectives on vitamin D, immune system, and chronic diseases. *Nutrition* 2011; 27:399–404.
15. Martins D, Wolf M, Pan D, Zadshir A, Tareen N, Thadhani R, Felsenfeld A, Levine B, Mehrotra R, Norris K. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey. *Arch Intern Med* 2007; 167:1159–1165.
16. Witham MD, Nadir MA, Struthers AD. Effect of vitamin D on blood pressure: a systematic review and meta-analysis. *J Hypertens* 2009; 27:1948–1954.
17. Pittas AG, Chung M, Trikalinos T, Mitri J, Brendel M, Patel K, Lichtenstein AH, Lau J, Balk EM. Systematic review: Vitamin D and cardiometabolic outcomes. *Ann Intern Med* 2010; 152:307–314.
18. Pilz S, Tomaschitz A, Ritz E, Pieber TR. Vitamin D status and arterial hypertension: a systematic review. *Nat Rev Cardiol* 2009; 6:621–630.
19. Nielsen CB, Bech JN, Pedersen EB. Effects of prostacyclin on renal haemodynamics, renal tubular function and vasoactive hormones in healthy humans. A placebo-controlled dose-response study. *Br J Clin Pharmacol* 1997; 44:471–476.
20. Zittermann A. Vitamin D in preventive medicine: are we ignoring the evidence? *Br J Nutr* 2003; 89:552–572.
21. Griffin FC, Gadegbeku CA, Sowers MR. Vitamin D and subsequent systolic hypertension among women. *Am J Hypertens* 2011; 24:316–321.
22. Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S, Meunier PJ. Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int* 1997; 7:439–443.
23. Durazo-Arvizu RA, Dawson-Hughes B, Sempos CT, Yetley EA, Looker AC, Cao G, Harris SS, Burt VL, Carriquiry AL, Picciano MF. Three-phase model harmonizes estimates of the maximal suppression of parathyroid hormone by 25-hydroxyvitamin D in persons 65 years of age and older. *J Nutr* 2010; 140:595–599.
24. Witham MD, Dove FJ, Dryburgh M, Sugden JA, Morris AD, Struthers AD. The effect of different doses of vitamin D(3) on markers of vascular health in patients with type 2 diabetes: a randomised controlled trial. *Diabetologia* 2010; 53:2112–2119.
25. Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD. Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels. *Diabet Med* 2008; 25:320–325.
26. Pfeifer M, Begerow B, Minne HW, Nachtigall D, Hansen C. Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. *J Clin Endocrinol Metab* 2001; 86:1633–1637.
27. Krause R, Bühring M, Hopfenmüller W, Holick MF, Sharma AM. Ultraviolet B and blood pressure. *Lancet* 1998; 352:709–710.

28. Coats AJ, Radaelli A, Clark SJ, Conway J, Sleight P. The influence of ambulatory blood pressure monitoring on the design and interpretation of trials in hypertension. *J Hypertens* 1992; 10:385–391.
29. Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W, Kong J. Vitamin D: a negative endocrine regulator of the renin-angiotensin system and blood pressure. *J Steroid Biochem Mol Biol* 2004; 89-90:387–392.
30. Xiang W, Kong J, Chen S, Cao LP, Qiao G, Zheng W, Liu W, Li X, Gardner DG, Li YC. Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems. *Am J Physiol Endocrinol Metab* 2005; 288:E125–E132.
31. Li YC, Bergwitz C, Jüppner H, Demay MB. Cloning and characterization of the vitamin D receptor from Xenopus laevis. *Endocrinology* 1997; 138:2347–2353.
32. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, Lieben L, Mathieu C, Demay M. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev* 2008; 29:726–776.
33. Vaidya A, Forman JP, Hopkins PN, Seely EW, Williams JS. 25-Hydroxyvitamin D is associated with plasma renin activity and the pressor response to dietary sodium intake in Caucasians. *J Renin Angiotensin Aldosterone Syst* 2011; 12:311–319.
34. Forman JP, Williams JS, Fisher ND. Plasma 25-hydroxyvitamin D and regulation of the renin-angiotensin system in humans. *Hypertension* 2010; 55: 1283–1288.
35. Burgess ED, Hawkins RG, Watanabe M. Interaction of 1,25-dihydroxyvitamin D and plasma renin activity in high renin essential hypertension. *Am J Hypertens* 1990; 3:903–905.
36. Tiosano D, Schwartz Y, Braver Y, Hadash A, Gepstein V, Weisman Y, Lorber A. The renin-angiotensin system, blood pressure, and heart structure in patients with hereditary vitamin D-resistance rickets (HVDRR). *J Bone Miner Res* 2011; 26:2252–2260.
37. Fitzpatrick LA, Bilezikian JP, Silverberg SJ. Parathyroid hormone and the cardiovascular system. *Curr Osteoporos Rep* 2008; 6:77–83.
38. Rashid G, Bernheim J, Green J, Benchetrit S. Parathyroid hormone stimulates endothelial expression of atherosclerotic parameters through protein kinase pathways. *Am J Physiol Renal Physiol* 2007; 292:F1215–F1218.
39. Jorde R, Sundsfjord J, Haug E, Bonaa KH. Relation between low calcium intake, parathyroid hormone, and blood pressure. *Hypertension* 2000; 35:1154–1159.
40. Snijder MB, Lips P, Seidell JC, Visser M, Deeg DJ, Dekker JM, van Dam RM. Vitamin D status and parathyroid hormone levels in relation to blood pressure: a population-based study in older men and women. *J Intern Med* 2007; 261:558–565.
41. Lind L, Hvarfner A, Palmér M, Grimelius L, Akerström G, Ljunghall S. Hypertension in primary hyperparathyroidism in relation to histopathology. *Eur J Surg* 1991; 157:457–459.
42. Dickinson HO, Nicolson DJ, Cook JV, Campbell F, Beyer FR, Ford GA, Mason J. Calcium supplementation for the management of primary hypertension in adults. *Cochrane Database Syst Rev* 2006:CD004639.
43. Hulter HN, Melby JC, Peterson JC, Cooke CR. Chronic continuous PTH infusion results in hypertension in normal subjects. *J Clin Hypertens* 1986; 2:360–370.
44. St John A, Dick I, Hoad K, Retallack R, Welborn T, Prince R. Relationship between calcitrophic hormones and blood pressure in elderly subjects. *Eur J Endocrinol* 1994; 130:446–450.
45. He JL, Scragg RK. Vitamin D, parathyroid hormone, and blood pressure in the National Health and Nutrition Examination Surveys. *Am J Hypertens* 2011; 24:911–917.
46. Carthy EP, Yamashita W, Hsu A, Ooi BS. 1,25-Dihydroxyvitamin D3 and rat vascular smooth muscle cell growth. *Hypertension* 1989; 13:954–959.
47. Youn JC, Rim SJ, Park S, Ko YG, Kang SM, Choi D, Ha JW, Jang Y, Chung N. Arterial stiffness is related to augmented seasonal variation of blood pressure in hypertensive patients. *Blood Press* 2007; 16:375–380.
48. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. *Am J Clin Nutr* 2003; 77:204–210.
49. Jones G. Pharmacokinetics of vitamin D toxicity. *Am J Clin Nutr* 2008; 88:582S–586S.