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Confirmed hypertension and plasma 25(OH)D concentrations amongst elderly men

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Abstract. Burgaz A, Byberg L, Rautiainen S, Orsini N, Håkansson N, Ärnlöv J, Sundström J, Lind L, Melhus H, Michaëlsson K, Wolk A (Institute of Environmental Medicine, Karolinska Institiute, Stockholm; Department of Surgical Sciences, Section of Orthopaedics; Department of Public Health and Caring Sciences/Geriatrics, Uppsala University, Uppsala; School of Health and Social Studies, Dalarna University, Falun; and Department of Medical Sciences, Uppsala University Hospital, Uppsala, Sweden). Confirmed hypertension and plasma 25(OH)D concentrations among elderly men. *J Intern Med* 2010; doi: 10.1111/j.1365-2796.2010.02309.x.

Objectives. The results of experimental studies suggest that vitamin D deficiency activates the renin–angiotensin system and predisposes to hypertension. Results of previous epidemiological studies investigating the association between 25-hydroxyvitamin D [25(OH)D] status and hypertension have not been consistent, perhaps because of their sole reliance on office blood pressure (BP) measurements leading to some misclassification of hypertension status. No previous studies have examined the association between 25(OH)D status and confirmed hypertension assessed with both office and 24-h BP measurements.

Design. In this cross-sectional study, we investigated 833 Caucasian men, aged 71 ± 0.6 years, to determine the association between plasma 25(OH)D concentrations, measured with high-pressure liquid chromatography mass spectrometry, and the prevalence of hypertension. We used both supine office and 24-h BP measurements for classifying participants as normotensive or confirmed hypertensive; participants with inconsistent classifications were excluded.

Results. In a multivariable adjusted logistic regression model, men with 25(OH)D concentrations <37.5 nmol L^{-1} had a 3-fold higher prevalence of confirmed hypertension compared to those with ≥37.5 nmol L^{-1} 25(OH)D (odds ratio = 3.3, 95% CI: 1.0–11.0).

Conclusions. Our results show that low plasma 25(OH)D concentration is associated with a higher prevalence of confirmed hypertension.

Keywords: 25-hydroxyvitamin D concentrations, blood pressure, confirmed hypertension, vitamin D.

Introduction

Hypertension is one of the most important risk factors for cardiovascular disease, which is the major cause of morbidity and mortality worldwide [1, 2]. Over the last decade, accumulating evidence has indicated that the concentration of 25-hydroxyvitamin D [25(OH)D] in the blood is inversely associated with blood pressure (BP) [3]. With regard to the underlying mechanisms of this association, clinical studies have shown that 25(OH)D suppresses the activity of the hormone renin, which in high concentrations can cause raised BP [4, 5]. Other potential mechanisms could be related to the effects of vitamin D on the cells of the vessel wall. These cells express the vitamin D receptor as well as 1α-hydroxylase, which converts 25(OH)D into the active form [6].

The majority of previous studies presented only correlations between plasma 25(OH)D concentration (which regulates the active compound 1,25-dihydroxycholecalciferol ($1,25(OH)_2D$) [7]) and office BP measurements [4, 8–16], with the exception of one study that used the more reliable measurement of

24-h BP [17]. Analytical epidemiological studies investigating associations between 25(OH)D concentrations and hypertension status also used office BP measurements [18–29]. Ambulatory measurements of BP over a 24-h period offer more precise information to classify subjects into a hypertensive or normotensive group than can be obtained from a conventional office BP assessment [30–32]. Indeed, previous studies have established that 24-h BP measurements are a more powerful predictor of cardiovascular morbidity and mortality than office BP, independent of other established cardiovascular risk factors [33, 34]. No previous studies have reported the association between 25(OH)D status and confirmed hypertension assessed by a combination of office and 24-h BP measurements. Use of this combination of BP measurements allows hypertension status to be assessed with a much higher degree of accuracy [33, 35–37].

The aim of this cross-sectional study was to investigate the relation between plasma 25(OH)D concentration and the prevalence of confirmed hypertension in a community-based sample of elderly Caucasian men. We measured 25(OH)D concentration using high-pressure liquid chromatography (HPLC) mass spectrometry (MS), which is considered the gold standard method [38, 39]. We used both supine office and 24-h BP measurements for classifying participants as normotensive or hypertensive. Only men who were consistently classified into one of the two categories according to the two BP assessments were included in analyses.

Material and methods

Study population

The study population was a subgroup of the ongoing community-based Uppsala Longitudinal Study of Adult Men (ULSAM), which was initiated in 1970 and originally included 2322 men. At the time of initiation, all 50-year-old men who were born between 1920 and 1924 and living in Uppsala, central Sweden, were invited to participate (for details, see http://www.pubcare.uu.se/ULSAM). There was an 82% response rate for the original cohort that focused on identifying metabolic risk factors for cardiovascular disease. The cohort was reinvestigated 21 years later (1991–1995). During the 21-year period, 422 men died and 219 moved out of the Uppsala region. Of the 1681 men invited, 1221 (73%) participated in this follow-up study (1991–1995). Men not participating in the follow-up did not vary from those who did with regard to body mass index (BMI) at age 50 (25.1 vs. 24.8 kg m^{-2}, respectively, $P = 0.11$).

Ethics

All participants provided written informed consent and the Ethics Committee of Uppsala University approved the study.

Measurements

Systolic (SBP) and diastolic blood pressure (DBP) (office BP) were measured twice in the right arm with the subject in the supine position after a period of rest of at least 10 min using a calibrated mercury sphygmomanometer to the nearest mmHg, and the average of the two recordings was used. SBP and DBP were defined as Korotkoff phases I and V, respectively.

Twenty-four-hour ambulatory SBP and DBP (24-h BP) were recorded using Accutracker II equipment (SunTech Medical Instruments Inc, Raleigh, NC, USA). The device was attached to the participant's nondominant arm by a skilled laboratory technician. BP was recorded every 20 or 30 min between 6 AM and 11 PM and every 20 or 60 min between 11 PM and 6 AM as previously described [40]. Arterial hypertension was defined as SBP >140 mmHg and/or DBP >90 mmHg using office BP measurements [28, 41] and as SBP >130 mmHg and/or DBP >85 mmHg using 24-h BP measurements [42]. Participants who were taking anti-hypertensive medication were classified as hypertensive.

Plasma 25(OH)D (D_2 and D_3) was determined with HPLC atmospheric pressure chemical ionization (APCI) MS at Vitas (Oslo, Norway; http://www.vitas.no). HPLC was performed with an HP 1100 liquid chromatograph (Agilent Technologies, Palo Alto CA, USA) interfaced by APCI to an HP MS operated in single-ion monitoring mode. This method has a recovery rate of 95%, is linear from 5 to 400 nmol L^{-1} and the limit of detection is 1–4 nmol L^{-1}. The reported inter-assay coefficients of variation (CV) for this method were 7.6% at an 25(OH)D concentration of 47.8 nmol L^{-1} and 6.9% at 83.0 nmol L^{-1}. The assay is accredited by the Vitamin D External Quality Assessment Scheme (DEQAS). Samples for 25(OH)D analysis had been stored at –70°C for up to 15 years; 25(OH)D is stable in stored frozen plasma [43]. The following clinical tests were carried out directly after blood collection. Calcium in serum (S-calcium) was

measured using spectrophotometry and a complexometric method with *o*-cresol-ftalein; CV = 1.6% at the 2.43 mmol L^{-1} level (reference 2.20–2.60 mmol L^{-1}). Serum phosphate (S-phosphate) was measured using spectrophotometry and a complexometric method with ammoniummolybdenum; CV = 1.9% at the 1.84 mmol L^{-1} level (reference 0.74–1.54 mmol L^{-1}). Creatinine in serum (S-creatinine) was measured with spectrophotometry using Jaffe's reaction; CV = 2.1% at the 147 μmol L^{-1} level (reference 60–106 μmol L^{-1}). Serum uric acid (S-uric acid) was measured by spectrophotometry. The uric acid is oxidized by uricase to peroxide which in turn generates quinonamine, which is measured at 546 nm; CV = 3.8% at the 427 μmol L^{-1} level (reference for men 160–450 μmol L^{-1}). S-calcium, S-phosphate, S-creatinine and S-uric acid were measured with a Hitachi 717 or 911 analyser (Japan) using reagents from Boehringer Mannheim.

Data regarding ongoing medical treatment and social characteristics were collected with a self-administered questionnaire. Leisure time physical activity was estimated using four questions. Based on these questions, participants were divided into four different physical activity categories: sedentary, moderate, regular and athletic, as described and validated previously [44]. Alcohol intake was assessed in grams per day [45] and body weight was measured to the nearest 0.1 kg and height to the nearest whole centimetre. BMI was calculated as weight divided by height squared. Information on smoking habits was retrieved from detailed interview reports, and smoking status was categorized as current, former or never smoker.

Of the 1221 men participating in the 1991–1995 follow-up investigation, 1011 had valid measurements of plasma 25(OH)D concentration, supine office BP and ambulatory 24-h BP and information on BMI, physical activity and alcohol consumption. Office BP and 24-h BP were used to classify men into normotensive and confirmed hypertensive categories. Men whose hypertension status was not consistent between the two measurement methods were excluded (n = 178), thus a total of 833 subjects were included in the analysis.

Statistical analyses

Unconditional logistic regression was used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) for the association between 25(OH)D concentrations and confirmed hypertension.

First, we compared two categories of plasma 25(OH)D concentrations: <37.5 and ≥37.5 nmol L^{-1}. Then, plasma 25(OH)D concentrations were divided into five categories according to cut-offs used in previous studies: <37.5, 37.5–49.9, 50.0–74.9, 75.0–100.0 and >100.0 nmol L^{-1} (concentration in nmol L^{-1} divided by 2.496 equals concentration in ng mL^{-1}) [46, 47]. The largest group (50.0–74.9 nmol L^{-1}), representing 49% of the study population, was used as the reference group.

We did not adjust for age because all participants were almost the same age (±0.6). A multivariable model was adjusted for BMI (continuous), physical activity (four categories) and alcohol intake (four categories, g d^{-1}); all of these factors are associated with BP [48] and with 25(OH)D status [14, 49, 50]. We adjusted for month of blood collection (12 categories, continuous) to take into account seasonal variability in 25(OH)D concentration. In an additional multivariable model, we further adjusted for S-calcium, S-phosphate, S-creatinine and S-uric acid (all continuous) to take into account renal function that is associated with BP and 25(OH)D through the renin–angiotensin system (RAS). Of all these variables, only alcohol intake indicated deviation from a linear pattern.

A P-value for nonlinearity was obtained by fitting a restricted cubic spline model with three knots at fixed percentiles (10%, 50%, 90%) and testing whether the coefficient of the cubic spline is equal to zero.

Two-tailed significance values of $P < 0.05$ were regarded as statistically significant. All analyses were performed using sas statistical software, version 9.2 (SAS institute Inc., Cary, NC, USA).

Results

The characteristics of the study participants, classified as normotensive and confirmed hypertensive, are shown in Table 1. The study population was investigated at 70 years of age (range 69.5–74.1). Plasma 25(OH)D concentrations were on average 3% lower in the participants with confirmed hypertension compared to the normotensive group. The prevalence of low 25(OH)D concentrations (<37.5 nmol L^{-1}) was 2.5 times higher in the confirmed hypertensive group than in the normotensive group. Mean BMI and alcohol intake were also higher in the confirmed hypertensive group than in the normotensive group. BMI (but no other variables) was statistically significantly different in the two groups ($P < 0.0001$).

Table 1 *Characteristics of the study participants (833 elderly men) according to blood pressure*

	Normotensive, n = 184	Confirmed hypertensive, n = 649
25(OH)D concentrations, mean ± SD nmol L^{-1}	70 ± 17	68 ± 18
25(OH)D concentrations, n (%)		
<37.5 nmol L^{-1}	3 (2)	32 (5)
≥37.5–49.9 nmol L^{-1}	20 (11)	71 (11)
50.0–74.9 nmol L^{-1}	91 (49)	319 (49)
75.0–100.0 nmol L^{-1}	65 (35)	205 (32)
>100.0 nmol L^{-1}	5 (3)	22 (3)
Blood pressure, mm Hg, mean ± SD		
Office		
Systolic	126 ± 8	154 ± 16
Diastolic	76 ± 6	87 ± 9
24-h		
Systolic	119 ± 6	141 ± 15
Diastolic	70 ± 4	79 ± 8
Body mass index (kg m^{-2}), mean ± SD	24.9 ± 2.7	26.7 ± 3.5
Alcohol intake (g d^{-1}), mean ± SD	5.6 ± 6.2	6.6 ± 7.6
Leisure time physical activity, n (%)		
Sedentary	5 (4)	26 (4)
Moderate	59 (32)	231 (36)
Regular + Athletic	120 (65)	392 (60)

25(OH)D, 25-hydroxyvitamin D.

The association between plasma 25(OH)D concentration and hypertension is shown in Table 2. Men with a 25(OH)D concentration <37.5 nmol L^{-1} had a 3-fold higher prevalence of hypertension compared to those with a concentration ≥37.5 nmol L^{-1}. This 3-fold higher prevalence remained in the multivariable model adjusted for BMI, physical activity, alcohol intake and month of blood collection (Table 2), as well as for clinical factors (S-calcium, S-phosphate, S-creatinine and S-uric acid) (OR = 2.9, 95% CI: 0.9–10.0). Further adjustment for smoking did not change the OR.

In a multivariable model based on the five categories of 25(OH)D concentration (Table 2), men with the lowest 25(OH)D concentrations (<37.5 nmol L^{-1}) had an OR of 3.2 (95% CI: 1.0–11.1) compared to the reference group (50–74.9 nmol L^{-1}). In the group with the highest concentrations (>100 nmol L^{-1}), the prevalence of hypertension was nonsignificantly increased by 40%. However, this potential U-shaped relationship was not confirmed by a formal test for curvature of the association (P = 0.39).

Discussion

In this community-based cross-sectional study of 70-year-old men, we observed a 3-fold higher prevalence of confirmed hypertension amongst men with low 25(OH)D concentrations (<37.5 nmol L^{-1}, 15 ng mL^{-1}) compared to those with levels ≥37.5 nmol L^{-1}.

A possible mechanism for the association between a low concentration of 25(OH)D (which regulates the active compound $1,25(OH)_2D$ [7]) and hypertension may be through activation of the RAS. It has been shown that $1,25(OH)_2D$, directly and negatively, regulates *renin* gene transcription through a vitamin D receptor-mediated mechanism [51]. As a potent negative regulator, vitamin D may play a key role in preventing the over-stimulation of the RAS. Other possible mechanisms may be related to the effects of vitamin D on the cells of the vessel wall (endothelial cells, vascular smooth muscle cells and macrophages), all of which express the vitamin D receptor as well as 1α-hydroxylase, which converts 25(OH)D to $1,25(OH)_2D$ [6]. It has also been speculated that vitamin D has potential nephroprotective effects and might be used therapeutically to minimize the influence of diseases that affect the kidney [52].

Therefore, an optimal level of circulating $1,25(OH)_2D$, which is regulated by 25(OH)D concentrations, is thought to be crucial for a normal level of BP [51].

Of interest, the ecological Intersalt Study, which examined more than 10 000 participants from around the world, showed that office BP was statistically significantly positively associated with distance from the equator, which was used as a proxy for ultraviolet B radiation exposure-dependent vitamin D status [53, 54]. Most [8, 9, 11, 14, 17], but not all [15, 16], recent studies also found inverse correlations between 25(OH)D concentrations and office BP. Although Sweden is a country with limited sunlight during the winter, findings show that vitamin D insufficiency is rare in community-dwelling elderly people [55]. These results are consistent with previous comparisons of serum vitamin D concentrations amongst populations in Europe, which have shown

Table 2 *Low plasma 25-hydroxyvitamin D [25(OH)D] concentrations and odds ratios (ORs) of confirmed hypertension [Office BP: systolic blood pressure (SBP) >140 mmHg and/or diastolic blood pressure (DBP) >90 mmHg; 24-h BP: SBP >130 mmHg and/or DBP >85 mmHg] amongst elderly men*

	Normtensive *n* (%)	Confirmed hypertensive *n* (%)	OR (95%CI)[a]	OR (95%CI)[b]
25(OH)D concentrations				
Two categories				
≥37.5 nmol L^{-1} (≥15 ng mL^{-1})	181 (23)	617 (77)	1.0 (ref)	1.0 (ref)
<37.5 nmol L^{-1} (<15 ng mL^{-1})	3 (9)	32 (91)	3.1 (0.9–10.3)	3.3 (1.0–11.0)
Five categories				
<37.5 nmol L^{-1} (<15 ng mL^{-1})	3 (9)	32 (91)	3.0 (0.9–10.2)	3.2 (1.0–11.1)
37.5–49.9 nmol L^{-1} (15–20 ng mL^{-1})	20 (22)	71 (78)	1.0 (0.6–1.7)	0.9 (0.5–1.6)
50–74.9 nmol L^{-1} (20–30 ng mL^{-1})	91 (22)	319 (78)	1.0 (ref)	1.0 (ref)
75–100 nmol L^{-1} (30–40 ng mL^{-1})	65 (24)	205 (76)	0.9 (0.6–1.3)	1.0 (0.7–1.4)
>100 nmol L^{-1} (>40 ng mL^{-1})	5 (19)	22 (81)	1.3 (0.5–3.4)	1.4 (0.5–3.9)

[a]Univariate regression. [b]Adjusted for body mass index (kg m^{-2}, continuous), physical activity (sedentary, moderate, regular, athletic), alcohol intake (four categories, g d^{-1}) and month when blood sample was collected (12 categories, continuous).

that the highest levels are found in the Nordic countries [56, 57]. In a recent study of Swedish twins, it has been shown that genetic factors are an important determinant of vitamin D status, and the key genetic effect appears to be on the cutaneous synthesis of vitamin D [58]. The results of another recent European study also suggest that genetic factors are a component of vitamin D insufficiency [59].

Our results, which are based on a reliable diagnosis of confirmed hypertension (i.e. a combination of 24-h BP and supine office BP measurements), are in agreement with the majority of previous analytical epidemiological studies of hypertension as diagnosed by office BP measurement. These studies reported findings between 25(OH)D concentrations and hypertension ranging from statistically significant inverse association [18–24] and nonsignificant inverse association [26, 27] to nonsignificant positive association [28, 29].

There is no consensus on the optimal range of vitamin D concentrations for good general health [60, 61]. Our study indicates that 25(OH)D levels higher than 37.5 nmol L^{-1} are required for normal BP in men. The authors of the Health Professionals Follow-Up Study of 38 388 men also concluded that the 25(OH)D concentration required for normal BP was at least 37.5 nmol L^{-1} [19]. In that study, however, serum 25(OH)D concentrations were predicted from questionnaire-based information, and hypertension status was self-reported. An expert panel has recently recommended a target range for 25(OH)D concentrations of 75–100 nmol L^{-1} (30–40 ng mL^{-1}) to reduce chronic disease including hypertension [62]. Our results may suggest a higher (although not statistically significant) prevalence of confirmed hypertension in the group with 25(OH)D concentrations above 100 nmol L^{-1}. This observation should be considered in the context of other recent studies of Nordic populations reporting a U-shaped association between 25(OH)D concentrations and tuberculosis [63], prostate cancer [64] and total cancer mortality [65] as well as a study reporting a U-shaped association with the ageing process in mice [66]. All these results taken together suggest that there may be an optimal range of vitamin D status and that 25(OH)D concentrations above this optimal level might have adverse effects on health. Further studies are warranted to study the association at very high 25(OH)D concentrations and in different populations because genetic factors might be involved.

Meta-analyses of vitamin D supplementation have reported weak evidence to support an effect to lower BP [67, 68] and reduce total mortality [69].

The main strengths of our study are the community-based design, the homogenous population with regard to gender and ethnicity and the narrow age-range of the participants. Another strength is that 25(OH)D concentrations are based on the gold

standard procedure of HPLC-MS analysis [38, 39]. All diagnoses were based only on confirmed hypertension. By using two techniques to measure BP (supine office and 24-h BP), we have reduced the likelihood of misclassification of subjects into the normotensive and confirmed hypertensive groups. All models were adjusted for potentially important confounders including month of blood sample collection and circulating biomarkers of renal function. However, we cannot entirely rule out the possibility that the associations we observed were partly attributed to unmeasured factors or to residual confounding.

A limitation of the study was its cross-sectional design and thus a cause-and-effect relationship between 25(OH)D concentrations and hypertension is uncertain. Other limitations include the low numbers of men with plasma 25(OH)D concentrations <37.5 or >100 nmol L^{-1}, so that we were unable to analyse the effect of even lower or higher 25(OH)D concentrations. The fact that only Caucasian men participated in our study limits the generalizability of the results to other ethnic groups and women.

In conclusion, our results show that low plasma 25(OH)D concentrations (<37.5 nmol L^{-1}) are associated with a higher prevalence of confirmed hypertension. Both longitudinal and interventional studies, with adequate measurements of both 25(OH)D concentrations and blood pressure, are needed to define the optimum vitamin D status.

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Conflict of interest statement

No conflict of interest was declared.

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