

Waist circumference modifies the association between serum 25(OH)D and systolic blood pressure: results from NHANES 2001–2006

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Objective: Results on the association between vitamin D and blood pressure are conflicting and little is known about how their relationship may be affected by obesity. Thus, we explored whether waist circumference modified the association between serum 25-hydroxyvitamin D (25(OH)D) and blood pressure in participants of the U.S. National Health and Nutrition Examination Surveys 2001–2006.

Methods: We included 10 331 nonpregnant participants aged 20 years or older. The association of serum 25(OH)D with systolic and diastolic blood pressure, in the total sample and stratified by waist circumference category (abdominal overweight: 80 to <88 cm in females / 94 to <102 cm in males; abdominal obesity: ≥88 cm in females/ ≥102 cm in males), was examined using multiple linear regression. Effect modification by waist circumference was assessed through a cross-product interaction term between 25(OH)D category and waist circumference category.

Results: Waist circumference significantly modified the inverse association between 25(OH)D and systolic blood pressure (SBP) (*P* value for interaction: 0.09). A stronger association of 25(OH)D levels below 15 ng/ml (reference: ≥30 ng/ml) with SBP was found in abdominally obese ($\beta = 3.5$ mmHg) than in abdominally overweight ($\beta = 2.0$ mmHg) and normal waist participants ($\beta = 1.2$ mmHg), but this interaction was only significant in participants without antihypertensive treatment. No significant effect modification was found for diastolic blood pressure.

Conclusion: Results from this large, cross-sectional sample suggest that the association between 25(OH)D and SBP is stronger in individuals with abdominal obesity than in those with a normal waist or with abdominal overweight.

Keywords: blood pressure, interaction, NHANES, obesity, vitamin D, waist circumference

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ACE, angiotensin converting enzyme; BMI, body mass index; DBP, diastolic blood pressure; GFR, glomerular filtration rate; NHANES, National Health and Nutrition Examination Surveys; RAS, renin–angiotensin system; SBP, systolic blood pressure

25-hydroxyvitamin D (25(OH)D) [2], the major circulating form of vitamin D, which is traditionally used as a measure of vitamin D status. Beyond its classical functions [3], the effect of vitamin D on health, especially its role in non-skeletal health, is an issue of controversy [4]. In observational studies, 25(OH)D levels are consistently inversely associated with systolic blood pressure (SBP) [5–8] and diastolic blood pressure (DBP) [7,8] levels as well as with the risk for hypertension [9,10]. Similar results were found in a Mendelian randomization study for genetically instrumented 25(OH)D levels [11]. By contrast, although results from individual clinical trials are ambiguous, recent meta-analyses did not demonstrate a significant effect of vitamin D supplementation on blood pressure [12,13].

Nonetheless, a relationship between vitamin D and blood pressure is biologically plausible. Results from human, animal and in-vitro studies suggest that vitamin D is a negative endocrine regulator of the renin–angiotensin system (RAS), a regulatory cascade essential in the regulation of blood pressure [14,15]. Other potential mechanisms include a regulation of the vascular smooth muscle contractility by vitamin D via calcium [16] as well as a role of vitamin D in the development of insulin resistance [17] which is involved in the pathogenesis of hypertension [18].

Accordingly, the relationship between vitamin D and blood pressure is complex and may be moderated by another, closely related factor. Obesity is a well established risk factor for hypertension [19]. Further, 25(OH)D levels are known to be low in overweight and obese individuals [20] and inversely associated with body mass index (BMI) [21], which is, among other factors, likely due to adipose sequestration [20]. Excess adipose tissue plays a role in the development of insulin resistance [22] and being obese was found to significantly modify the association between

Journal of Hypertension 2016, 34:637–645

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Received 12 August 2015 Revised 30 September 2015 Accepted 2 December 2015
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DOI:10.1097/HJH.0000000000000840

INTRODUCTION

Low vitamin D levels are common worldwide [1]. Between 2001 and 2006, 32% of the U.S. population had inadequate levels of less than 20 ng/ml of serum

25(OH)D and insulin resistance [23]. Further, obesity is associated with an over-activated RAS [24]. Results from cross-sectional studies show that increasing BMI and adiposity partly mediate the association of low 25(OH)D with high blood pressure [5,7,25]. However, the presence of an interaction between 25(OH)D and obesity on blood pressure has rarely been examined. We hypothesized that the association between 25(OH)D and blood pressure is stronger in obese individuals.

Thus, the objective of this study was to explore a possible effect modification by abdominal obesity on the association between serum 25(OH)D and blood pressure in adult participants of the cross-sectional U.S. National Health and Nutrition Examination Surveys (NHANES) 2001–2006.

METHODS

Ethics statement

All NHANES surveys were approved by the National Center for Health Statistics ethics review board. Participants provided informed consent prior to participation [26].

Study population

NHANES is an ongoing, cross-sectional survey of the resident civilian noninstitutionalized U.S. population, which is conducted by the Centers for Disease Control and Prevention's National Center for Health Statistics. A complex, four-

stage probability sampling design is used to select a nationally representative sample, with certain subgroups being oversampled to increase the reliability and precision of estimates (adolescents, adults aged 70 and over, non-Hispanic Blacks, Mexican Americans and persons with low-income in the NHANES waves 1999–2006). NHANES consists of home-administered questionnaires and a standardized physical examination in specially equipped mobile examination centers. A detailed description of NHANES, including information on sampling, interview, examination, and laboratory measurement of blood samples can be found elsewhere [26–28].

For this analysis, we pooled data from the NHANES waves 2001–2006. The response rates in these three waves varied from 79 to 84% for the interview and 76 to 80% for the examination [28]. We restricted our analysis to nonpregnant participants aged not less than 20 years with data on both interview and examination and excluded participants with missing information in any of the variables used for analyses, leading to a study population of 10 331 participants. The study population and the excluded participants are described in a flow chart in Fig. 1.

Physical examination

Height and weight were measured in adults dressed in underwear, disposable paper gowns, and foam slippers. A digital scale was used to measure weight to the nearest

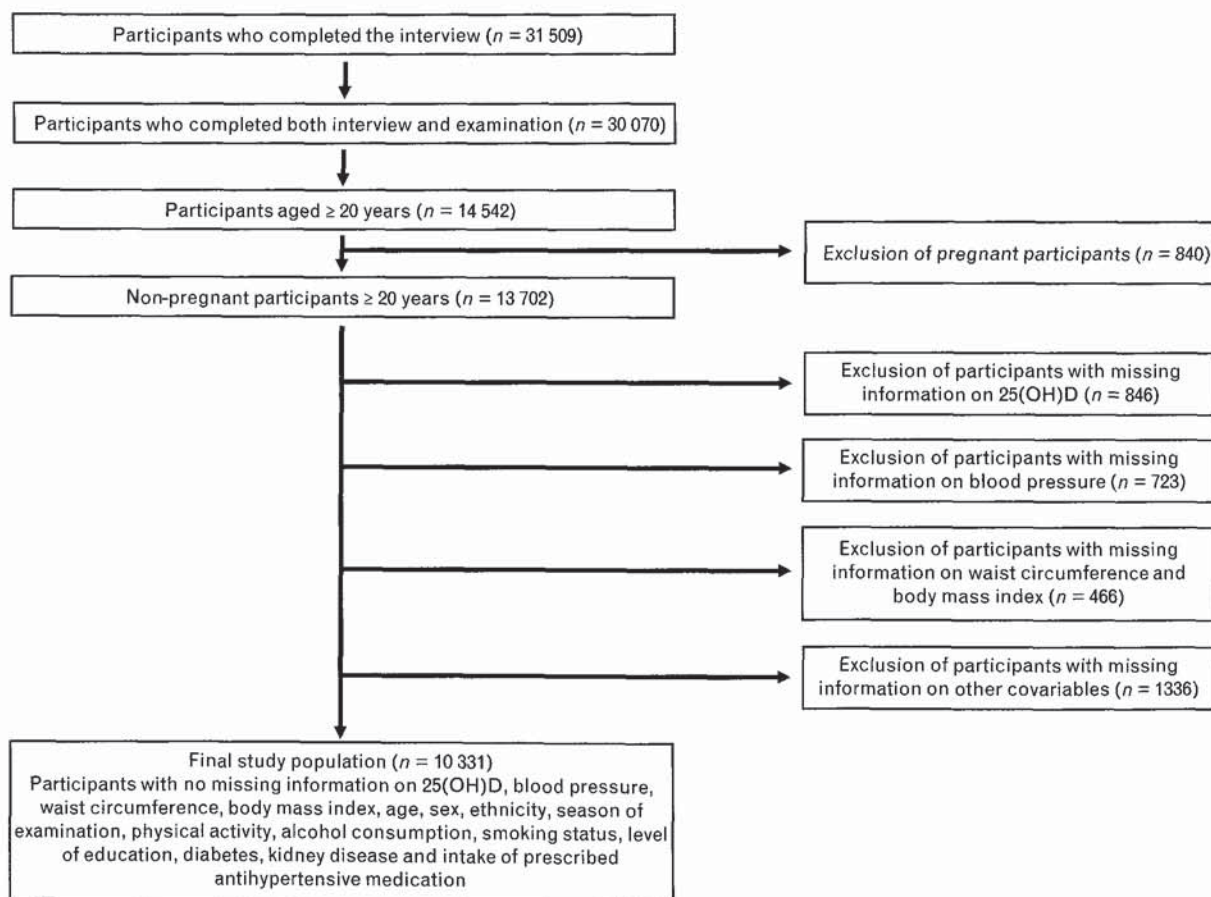


FIGURE 1 Description of the study population.

100 g, a fixed stadiometer to measure height to the nearest millimeter. BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured at the iliac crest to the nearest millimeter, using a steel tape [28].

Seated resting blood pressure was measured by a physician using a mercury sphygmomanometer (Baumanometer; WA Baum Co. Inc., New York, USA). Up to four measurements were obtained from each participant [28]. For this analysis, we calculated the average of the measurements, excluding the first measurement if more than one was available.

25-hydroxyvitamin D measurement

Blood was collected during the examination via venipuncture by certified phlebotomists from all participants, except those with hemophilia or those who received chemotherapy within the previous 4 weeks. About 89–92 ml (in 2001–2002), 104 ml (in 2003–2006) or 105 ml (2005–2006) were drawn from the examinee's arm using vacutainer tubes (Becton-Dickinson, Franklin Lakes, New Jersey, USA). The samples were centrifuged, aliquoted, and frozen to -20°C at the mobile examination centers before being transported to laboratories across the United States for analysis. Serum 25(OH)D was measured at the National Center for Environmental Health (CDC, Atlanta, Georgia, USA) using a radioimmunoassay kit (DiaSorin, Stillwater, Minnesota, USA) [28]. The sensitivity of this assay has been shown to be 1.5 ng/ml, the coefficients of variation for the years 2001–2006 varied between 4 and 13% [29]. As the 25(OH)D data from NHANES 2003–2006 was affected by assay drifts, we used the adjusted data, which was updated in November 2010 [30].

Covariable assessment

Information on age, sex, ethnicity, level of education, physical activity, smoking behavior, alcohol consumption, presence of diabetes as told by a doctor as well as current intake of prescribed antihypertensive medication were obtained from the questionnaire.

Participants who reported never having smoked 100 cigarettes during their lifetime were considered as never smokers. Those who reported having smoked at least 100 cigarettes during their lifetime but currently did not smoke were considered as former smokers, whereas those who reported smoking either every day or some days or reported smoking at least one cigarette per day were considered as current smokers. For alcohol consumption, we calculated the reported number of alcoholic beverages consumed per week and used the following categorization: 'no consumption' for women and men who reported no consumption of alcoholic beverages at all; 'moderate consumption' for women and men who reported consuming at least one but not more than 7 or 14 alcoholic beverages per week, respectively; 'heavy consumption' for women and men who reported consuming more than 7 or 14 alcoholic beverages per week, respectively. Information on physical activity applied to leisure time activities in the past 30 days. An activity which was performed for at least 10 min and caused heavy sweating or large increases in breathing or

heart rate was considered to be vigorous, an activity which was performed for at least 10 min and caused light sweating or slight to moderate increases in breathing or heart rate was considered to be moderate. For the classification, if participants performed both moderate and vigorous activities, the amount of vigorous activities defined the group allocation.

Chronic kidney disease was defined as an estimated glomerular filtration rate (GFR) below 60 ml/min per 1.73 m^2 [31] with GFR being estimated from serum creatinine using the CKD-EPI equation [32].

Statistical analysis

The associations of 25(OH)D with the continuous outcome variables SBP and DBP were examined using multiple linear regression. Waist circumference was used to operationalize abdominal obesity. As the relationship of blood pressure with 25(OH)D and waist circumference was not entirely linear, both variables were categorized. 25(OH)D levels were grouped into four categories ($<15\text{ ng/ml}$, 15 to $<20\text{ ng/ml}$, 20 to $<30\text{ ng/ml}$, $\geq 30\text{ ng/ml}$), based on cut-points used by the U.S. Institute of Medicine (16 and 20 ng/ml [3]) and by the U.S. Endocrine Society (20 and 30 ng/ml [33]). Normal waist was defined as a waist circumference $<80\text{ cm}$ in women or $<94\text{ cm}$ in men, abdominal overweight as a waist circumference of 80 to $<88\text{ cm}$ in women or 94 to $<102\text{ cm}$ in men, and abdominal obesity as a waist circumference $\geq 88\text{ cm}$ in women or $\geq 102\text{ cm}$ in men. 25(OH)D and waist circumference were also used as continuous variables in a sensitivity analysis.

First, the association of 25(OH)D with SBP and DBP in the total sample was assessed in models adjusted for waist circumference (main effect models). Further, effect modification by waist circumference was examined in two ways. Firstly, the main effect models were stratified by waist circumference category (stratified models). Secondly, a cross-product interaction term between 25(OH)D category and waist circumference category was added to the main effect models (interaction models). Possible differences in the interaction between 25(OH)D category and waist circumference category according to sex were examined by adding a three-way interaction term (25(OH)D category $\times$ waist circumference category $\times$ sex, together with the three respective cross-product terms) to the interaction models. In addition, the interaction models were stratified by intake of antihypertensive medication.

All models were adjusted for age, sex, waist circumference category, ethnicity, season of examination, physical activity, alcohol consumption, smoking status, level of education, diabetes, kidney disease and intake of prescribed antihypertensive medication. In a sensitivity analysis, both the main effect and the stratified models were additionally adjusted for BMI category to achieve a more stable representation of abdominal obesity by waist circumference. For this, normal weight was defined as a BMI $<25\text{ kg/m}^2$, overweight as a BMI of 25 to $<30\text{ kg/m}^2$, and obesity as BMI $\geq 30\text{ kg/m}^2$.

A significance level of 0.05 was used, except for the interaction terms. Estimates of interaction effects have

larger variances than estimates of additive effects and thus, the power of a statistical test to detect an interaction is lower [34]. To compensate for this, we chose a significance level of 0.1 for the interaction effects, which is considered more conventional for testing interactions [35].

All statistical analyses were carried out using SAS (Version 9.3; SAS Institute Inc., Cary, North Carolina, USA). To account for the complex design used in NHANES, SAS survey procedures (SURVEYMEANS, SURVEYREG) adjusted for sampling probability, stratum, and cluster effects were used to analyze the data. A combined 6-year examination weight for the three cycles was used [27]. The analytic sample was examined using the DOMAIN statement.

RESULTS

Descriptive analysis

The mean serum 25(OH)D level was 23.7 ng/ml, and levels ranged from 2.0 to 86.0 ng/ml. The characteristics of the study population in total and according to 25(OH)D category are displayed in Table 1. Mean SBP and DBP as well as mean BMI and waist circumference decreased with higher 25(OH)D levels. Participants with 25(OH)D levels below 15 ng/ml were more likely to be female, nonwhite, inactive, nondrinkers and current smokers. They were also more likely to have diabetes, to have reported intake of antihypertensive medication or to have been examined in the winter (November–April).

TABLE 1. Characteristics of the study population in total and by 25(OH)D category

		Total		25(OH)D category			
				<15 ng/ml	15 to <20 ng/ml	20 to <30 ng/ml	≥30 ng/ml
Characteristic	N	10 331	2258	2069	4146	1858	
SBP (mmHg)	Mean (SE)	121.9 (0.30)	125.2 (0.53)	122.5 (0.43)	121.6 (0.36)	119.8 (0.53)	
DBP (mmHg)	Mean (SE)	71.3 (0.22)	72.5 (0.43)	71.4 (0.32)	71.4 (0.28)	70.3 (0.33)	
BMI (kg/m ²)	Mean (SE)	28.1 (0.12)	30.3 (0.23)	29.3 (0.19)	27.9 (0.15)	26.1 (0.17)	
Waist circumference (cm)	Mean (SE)	96.8 (0.33)	100.7 (0.56)	99.6 (0.44)	96.7 (0.35)	92.1 (0.57)	
Age group							
20–29 years	%	18	19	20	17	18	
30–39 years	%	19	19	19	19	21	
40–49 years	%	23	25	20	24	21	
50–59 years	%	18	16	20	17	18	
60–69 years	%	11	11	12	12	10	
≥70 years	%	11	10	11	11	11	
Sex							
Male	%	50	42	50	53	48	
Female	%	50	58	50	47	52	
Ethnicity							
Non-Hispanic Black	%	10	35	13	4	1	
Non-Hispanic White	%	74	41	64	81	92	
Mexican-American	%	7	11	10	7	3	
Other ethnicity	%	9	13	13	8	4	
Physical activity (times/month)							
Vigorous (≥12)	%	19	13	15	19	26	
Vigorous (1–11)	%	16	11	14	16	18	
Moderate (≥12)	%	17	14	16	18	18	
Moderate (1–11)	%	15	13	16	16	14	
None	%	34	49	40	31	24	
Alcohol consumption							
Heavy	%	9	7	7	9	10	
None	%	29	38	33	28	22	
Moderate	%	63	55	60	63	68	
Smoking status							
Current	%	25	31	26	22	26	
Former	%	25	19	23	28	26	
Never	%	50	51	51	50	48	
Season of examination							
November–April	%	40	57	47	37	29	
May–October	%	60	43	53	63	71	
Level of education							
Less than 9th grade	%	6	8	8	6	4	
9–11th grade	%	11	17	12	10	8	
High-school graduate	%	26	27	26	25	27	
College or AA degree	%	31	31	32	31	32	
College graduate	%	26	18	23	29	29	
Kidney disease							
Yes	%	7	7	6	7	7	
No	%	93	93	94	93	93	
Diabetes							
Yes	%	7	12	10	6	5	
Borderline	%	1	1	2	1	1	
No	%	91	87	89	92	94	
Intake of antihypertensive medication							
Yes	%	21	24	23	20	17	
No	%	79	76	77	80	83	

25(OH)D, 25-hydroxyvitamin D; AA, associates degree; DBP, diastolic blood pressure; SBP systolic blood pressure; SE, standard error.

TABLE 2. Association between serum 25(OH)D and blood pressure in the total sample and stratified by WC category

		<i>N</i> = 10 331		SBP (mmHg)		DBP (mmHg)	
		25(OH)D category		β	<i>P</i>	β	<i>P</i>
Total sample ^a		<15 ng/ml	2258	2.7	0.001	1.6	0.01
		15 to <20 ng/ml	2069	0.6	0.27	0.6	0.14
		20 to <30 ng/ml	4146	0.4	0.34	0.6	0.08
		≥30 ng/ml	1858	Ref.		Ref.	
WC category		25(OH)D category		β	<i>P</i>	β	<i>P</i>
Normal ^b : <80 cm (women); <94 cm (men)		<15 ng/ml	481	0.9	0.57	1.0	0.33
		15 to <20 ng/ml	460	-0.4	0.72	0.6	0.49
		20 to <30 ng/ml	1125	0.6	0.45	0.5	0.40
		≥30 ng/ml	648	Ref.		Ref.	
Overweight ^b : 80 to <88 cm (women); 94 to <102 cm (men)		<15 ng/ml	372	1.4	0.32	1.0	0.32
		15 to <20 ng/ml	397	-0.5	0.65	0.3	0.80
		20 to <30 ng/ml	913	0.3	0.65	0.6	0.28
		≥30 ng/ml	444	Ref.		Ref.	
Obese ^b : ≥88 cm (women); ≥102 cm (men)		<15 ng/ml	1405	3.7	0.0008	2.1	0.02
		15 to <20 ng/ml	1212	1.2	0.13	0.8	0.20
		20 to <30 ng/ml	2108	0.2	0.83	0.7	0.01
		≥30 ng/ml	766	Ref.		Ref.	

Significant results are in *italics*. All models adjusted for age, sex, ethnicity, season of examination, physical activity, alcohol consumption, smoking status, level of education, diabetes, kidney disease and intake of prescribed antihypertensive medication. 25(OH)D, 25-hydroxyvitamin D; DBP, diastolic blood pressure; SBP systolic blood pressure; WC, waist circumference.

^aFully adjusted model without interaction term, adjusted for WC category.

^bFully adjusted model without interaction term, stratified by WC category.

Association between serum 25-hydroxyvitamin D and blood pressure

Table 2 presents the results of the multiple linear regression analyses of the association of 25(OH)D with SBP and DBP in the total sample and stratified by waist circumference category. In the main effect models, lower 25(OH)D levels were associated with a gradually higher mean SBP and DBP. 25(OH)D levels below 15 ng/ml, as compared with 25(OH)D levels ≥30 ng/ml, were significantly associated with a 2.7 mmHg higher SBP (*P* value: 0.001) and a 1.6 mmHg higher DBP (*P* value: 0.01). In the stratified models, a monotonic association of 25(OH)D with SBP and DBP was only found in abdominally obese participants. Further, the association of 25(OH)D levels below 15 ng/ml with SBP and DBP was only significant in abdominally obese participants, who had a 3.7 mmHg higher mean SBP (*P* value: 0.0008) and a 2.1 mmHg higher mean DBP (*P* value: 0.02) than participants with 25(OH)D levels ≥30 ng/ml.

The adjusted β -coefficients and 95% confidence intervals for the 25(OH)D categories from the interaction models are shown in Fig. 2. The association between 25(OH)D and SBP differed significantly according to waist circumference category (*P* value for interaction: 0.09). As compared with participants having 25(OH)D levels ≥30 ng/ml, abdominally obese participants with 25(OH)D levels <15 ng/ml had a 3.5 mmHg higher mean SBP, whereas the mean SBP in abdominally overweight and normal waist participants with 25(OH)D levels <15 ng/ml was only 2.0 and 1.2 mmHg higher. The association between 25(OH)D and DBP did not differ significantly according to waist circumference category (*P* value for interaction: 0.99). We found no significant differences in the interaction between

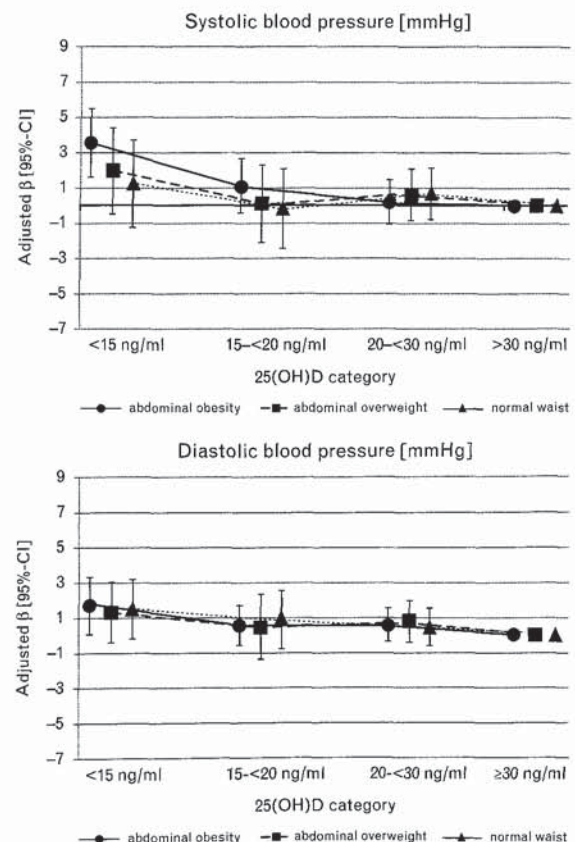


FIGURE 2 Adjusted β -coefficients and 95% confidence intervals for the 25(OH)D categories from the interaction models (all models adjusted for age, sex, ethnicity, season of examination, physical activity, alcohol consumption, smoking status, level of education, diabetes, kidney disease and intake of prescribed antihypertensive medication). Reference category: 25(OH)D ≥30 ng/ml.

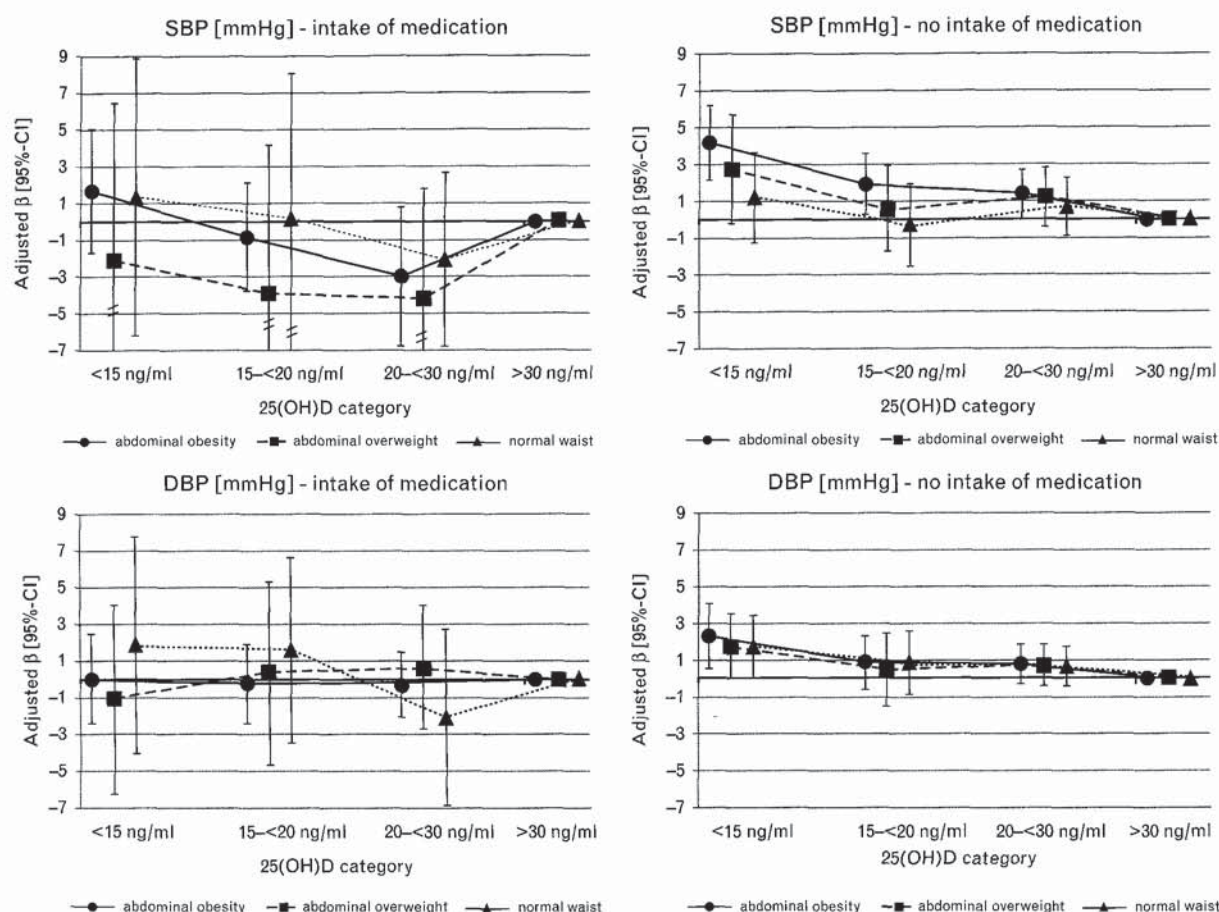


FIGURE 3 Adjusted β -coefficients and 95% confidence intervals for the 25(OH)D categories from the interaction models (all models adjusted for age, sex, ethnicity, season of examination, physical activity, alcohol consumption, smoking status, level of education, diabetes and kidney disease), stratified by intake of antihypertensive medication. Reference category: 25(OH)D ≥ 30 ng/ml.

25(OH)D category and waist circumference category according to sex (P values of the three-way interaction terms: 0.23 and 0.21 for SBP and DBP, respectively).

Subgroup analysis according to antihypertensive treatment

When the interaction models were stratified by intake of antihypertensive medication, a monotonic inverse association of 25(OH)D with SBP and DBP was found in abdominally obese participants who did not take medication, but not in participants who did (Fig. 3). Further, the interaction between 25(OH)D category and waist circumference category on SBP was significant in participants who did not take medication (P value for interaction: 0.09), but not in participants who did (P value for interaction: 0.97). No significant effect modification by waist circumference on the association between 25(OH)D and DBP was found for participants who did not take medication (P value for interaction: 0.27) or in participants who did (P value for interaction: 0.99).

Sensitivity analyses

Using both 25(OH)D and waist circumference as continuous variables in the interaction models confirmed the

results of the main analysis. The inverse association between 25(OH)D and SBP significantly strengthened with increasing waist circumference (P value for interaction: 0.07), whereas no significant difference with increasing waist circumference was observed for the inverse association between 25(OH)D and DBP (P value for interaction: 0.37). Further adjustment of both the main effect and the stratified models for BMI category had little influence (results not shown). Both the regression coefficients and the P values were only marginally attenuated.

DISCUSSION

In this large, nationally representative sample of adults aged 20 years or older, waist circumference significantly modified the inverse association between 25(OH)D and SBP, which was stronger in abdominally obese than in abdominally overweight and normal waist participants. The association between 25(OH)D and DBP did not significantly differ according to waist circumference category. After splitting the cohort by intake of antihypertensive medication, the significant effect modification by waist circumference on the association between 25(OH)D and SBP was only found in participants who did not take medication.

Our results on the association between 25(OH)D and blood pressure in the total sample are in line with another study using data from NHANES 2001–2006, in which 25(OH)D levels were significantly inversely associated with SBP but not with DBP [6]. We provide additional evidence for the finding that in models adjusted for obesity, the association of 25(OH)D with SBP is more pronounced than with DBP [5,6,36,37], although previously, 25(OH)D has also been found to be significantly associated with DBP [7,8,38]. Regarding our finding of a significant interaction between 25(OH)D and waist circumference on SBP, our results are in conflict with the only study that analyzed this interaction before. Sabanayagam *et al.* [39] studied 9215 adult NHANES III participants and found no significant interaction between 25(OH)D quartile and BMI category (*P* value for interaction: 0.56) on the association with prehypertension. However, this study is not totally comparable to ours, as Sabanayagam *et al.* excluded participants with hypertension, which are more likely to be obese. They also used BMI to operationalize obesity, which is less strongly correlated to the metabolically active visceral adipose tissue. Further, they compared participants with a BMI less than 25 kg/m² to participants with a BMI $\geq$ 25 kg/m², whereas we distinguished between three waist circumference groups and found the strongest association in abdominally obese participants.

Biologically, the RAS provides a potential link between vitamin D and blood pressure. The RAS cascade is activated when angiotensinogen from the liver is cleaved to angiotensin I by the enzyme renin, which is mainly produced by the kidneys [24]. Subsequently, angiotensin I is cleaved to angiotensin II, the main effector peptide of the RAS, by the angiotensin converting enzyme (ACE) [24]. Among other functions, angiotensin II is a potent vasoconstrictor of the arterioles and thus increases the blood pressure [14]. Consequently, elevated RAS activity is known to contribute to hypertension [16]. 1,25-dihydroxyvitamin, the active vitamin D metabolite, seems to downregulate RAS activity by suppressing the synthesis of renin, the rate-limiting component of the RAS [14]. 25(OH)D levels were found to be significantly inversely associated with plasma renin and angiotensin II concentrations in a large cohort of patients referred to coronary angiography [15]. In our study, an association between 25(OH)D and blood pressure was primarily found in participants who did not take antihypertensive medication, as compared with participants who did. In NHANES 2001–2006, ACE inhibitors were the second most commonly used class of antihypertensive drugs and the ACE inhibitor Lisinopril was the most commonly used individual antihypertensive drug [40]. Thus, 25(OH)D may have little to no additional effect in participants who take antihypertensive medication, as the RAS is already inhibited. In our study population, the proportion of participants who did take medication was small and consequently, the results for this group varied considerably (as indicated by the large 95% confidence intervals). Our results for these participants have thus to be interpreted with caution. However, a similar relationship was found in previous studies, in which the inverse association between 25(OH)D and blood pressure was

more pronounced in male participants not taking antihypertensive medication [36,38].

Apart from the systemic RAS, several organs, among them the adipose tissue, have a local RAS [24]. All RAS components are produced by adipocytes and these local RAS components contribute to the systemic RAS [41]. Hence, the local RAS is over-activated during obesity, which provides a link between obesity and cardiometabolic diseases [41]. Obesity was found to significantly modify the relationship between 25(OH)D and systemic RAS activity in hypertensive participants: although 25(OH)D was significantly inversely associated with RAS activity in obese individuals, no significant association was found in nonobese individuals [42]. Vaidya *et al.* propose two possible hypotheses as to why the effect of vitamin D on the RAS might be more pronounced during obesity [42]: the RAS-inhibitory effect of vitamin D may only be detectable during obesity, when the RAS is over-activated due to additional RAS components from the adipose tissue RAS; alternatively, the inhibition of the RAS by vitamin D could rather occur in the tissue RAS than in the systemic RAS. The latter hypothesis is supported by a small clinical trial, in which 1 month of 15 000 IU/day vitamin D₃ supplementation reduced end-organ tissue-RAS activity in obese participants, independent of the systemic RAS. The intervention also lowered mean arterial pressure by 3% [43]. Further, 25(OH)D levels were found to be positively associated with adiponectin, particularly in obese individuals, with BMI being a significant effect modifier in the 25(OH)D-adiponectin relationship, independent of circulating RAS activity [44]. Adiponectin is an adipokine which is known to be inversely associated with RAS activity [44] and blood pressure [45].

Another explanation for the significant effect modification by waist circumference on the association between 25(OH)D and SBP observed in this study could be insulin resistance. Kabadi *et al.* observed a strong interaction between abdominal obesity and insufficient 25(OH)D on insulin resistance [23], which in turn may play a role in the pathogenesis of hypertension [18].

Our study has several limitations. Due to the cross-sectional design, we cannot demonstrate causality or directionality. Further, due to the large proportion of non-Hispanic whites in the study population, our results were dominated by this ethnic group. Although we did adjust our models for a variety of covariables, there is still a possibility of residual confounding. Specifically, a higher 25(OH)D level could be the result of a healthy lifestyle involving increased sun exposure due to more frequent outdoor physical activity as well as a diet rich in vitamin D. Such a lifestyle, which would likely prevent high blood pressure, might not be sufficiently captured by adjustment for overall physical activity and smoking alone. Also, we did not adjust our models for serum parathyroid hormone, a possible confounder in the relationship between vitamin D and cardiovascular disease, because of the large amount of missing values in this variable. However, adjustment for parathyroid hormone had little effect on the association between 25(OH)D and blood pressure in data from NHANES 2001–2006 in another study [6]. Further, we had to exclude

3371 participants of the original sample because they had missing values in variables used for the analyses. As compared with these excluded participants, the participants in our study population were more likely to be non-Hispanic white (74 vs. 64%). They were also slightly more likely to be male (50 vs. 47%) and had a marginally smaller mean waist circumference and a lower mean BMI (96.8 cm and 28.1 kg/m², respectively) than the excluded participants (97.5 cm and 28.9 kg/m², respectively). These differences may have reduced the representativeness of our study population. However, we have assessed the interaction between 25(OH)D and waist circumference in models adjusted for age, sex and ethnicity in an enlarged study population, including those of the excluded 3371 participants who had information on blood pressure, 25(OH)D and waist circumference (SBP: *N*=11 846; DBP: *N*=11 768). As the results differed only marginally from those of the main analysis, we have reason to believe that excluding participants with missing values did not impair the internal validity of our results. Also, although we examined two different outcomes, we did not correct our analyses for multiple testing. As the evaluation of both SBP and DBP was planned and, as discussed above, we had a basis for expecting our results to be biologically plausible, we decided to not correct for multiple testing as suggested by Rothman [46]. However, this is a controversial question and it is important to note that, had we corrected our analyses for multiple testing, the interaction between 25(OH)D and waist circumference would have not been significant for SBP. Further, the association between 25(OH)D and blood pressure was only modest. Compared with participants having 25(OH)D levels ≥ 30 ng/ml, the mean SBP in abdominally obese patients with very low vitamin D levels was only 3.5 mmHg higher. Given that the target for most patients is a SBP below 140 mmHg, this effect is small for the individual. However, a 2 mmHg decrease in the mean SBP levels of the general population would involve a lower cardiovascular mortality of 7–10% [47]. Finally, we were not able to account for a possible circadian rhythm of 25(OH)D levels, as the exact time of the venipuncture for each participant is not released in NHANES. However, no significant difference was found in mean 25(OH)D levels between participants who were examined in the morning after an overnight fast and participants who were examined in the afternoon or evening, and the distributions of 25(OH)D levels were rather similar.

In conclusion, our results suggest that individuals with abdominal obesity who are vitamin D deficient are even more likely to have a high blood pressure than their counterparts with a normal waist or with abdominal overweight. As 25(OH)D levels are known to be especially low in the obese [20], this group might be particularly disadvantaged. Further research is needed to establish whether the associations found in this study are causal and whether vitamin D supplementation can be used to prevent hypertension in obese adults. At this point, monitoring of 25(OH)D levels should be emphasized for obese individuals and this group might be considered as a special target group for strategies to enhance vitamin D levels.

ACKNOWLEDGEMENTS

The authors would like to thank the Centers for Disease Control and Prevention, Hyattsville, Maryland, USA, for making the data available for analysis.

This work was supported by the Kompetenznetz Adipositas (Competence Network Obesity) funded by the German Federal Ministry of Education and Research (FKZ: 01GI1121B).

Conflicts of interest

There are no conflicts of interest.

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Reviewers' Summary Evaluations

Reviewer 1

Strengths include: (1) population-based sample (NHANES), (2) thoughtful, hypothesis-based analysis of data based on an appropriate literature review, (3) clear presentation of findings supporting a relationship between Vit D and blood pressure in abdominally obese subjects that was not observed in abdominally overweight and abdominally normal weight individuals.

Weaknesses include: (1) a cross-sectional study, (2) unknown confounders of the relationship between Vit D, blood pressure and waist circumference, (3) a marginally significant interaction of waist circumference as a modifier of the relationship between 25(OH)D and blood pressure.

Reviewer 2

The influence of obesity on the association between vitamin D and blood pressure has rarely been examined. The NHANES 2001–2006 shows that vitamin D deficient individuals with obesity are even more likely to have a high blood pressure than those with a normal waist or with abdominal overweight. This is an important and valuable study. However, there are limitations. Due to a cross-sectional study, the reverse causality cannot be excluded. A number of useful data are not provided: possible circadian rhythm of 25(OH)D levels, use of serum parathyroid hormone as a confounding variable, and exposure of sunlight time.