

## Plasma 25-Hydroxyvitamin D and Regulation of the Renin-Angiotensin System in Humans

John P. Forman, Jonathan S. Williams, Naomi D.L. Fisher

**Abstract**—Vitamin D regulates the renin-angiotensin system (RAS) in experimental animals, but corresponding human data are limited. We examined the relation between plasma 25-hydroxyvitamin D and elements of the RAS in 184 normotensive individuals in high sodium balance; these included circulating levels of plasma renin activity and angiotensin II (Ang II) and the renal plasma flow response to infused Ang II, which is an indirect measure of the intrinsic RAS activity in the kidney. Compared with individuals with sufficient 25-hydroxyvitamin D levels ( $\geq 30.0$  ng/mL), those with insufficiency (15.0 to 29.9 ng/mL) and deficiency ( $< 15.0$  ng/mL) had higher circulating Ang II levels ( $P$  for trend=0.03). Moreover, those with vitamin D deficiency had significantly blunted renal plasma flow responses to infused Ang II (mean decrease of 115 mL/min per 1.73 m<sup>2</sup> in renal plasma flow versus 145 mL/min per 1.73 m<sup>2</sup> among those with sufficient vitamin D levels;  $P$  for trend=0.009). Although plasma renin activity was higher among individuals with insufficient levels of vitamin D, the result was not statistically significant. These data suggest that low plasma 25-hydroxyvitamin D levels may result in upregulation of the RAS in otherwise healthy humans. (*Hypertension*. 2010;55:1283-1288.)

**Key Words:** vitamin D ■ renin-angiotensin system ■ hypertension ■ epidemiology ■ human

Observational studies strongly support an inverse association between plasma 25-hydroxyvitamin D (25[OH]D) concentration and blood pressure, as well as hypertension.<sup>1-14</sup> A major proposed mechanism linking vitamin D with hypertension involves vitamin D-mediated suppression of the renin-angiotensin (Ang) system (RAS), yet data derive mostly from in vitro and animal studies.<sup>15-19</sup>

Human investigation of the association between vitamin D and the RAS has been scant. Resnick et al<sup>20</sup> originally reported that plasma renin activity (PRA) and 1,25-dihydroxyvitamin D (1,25[OH]<sub>2</sub>D) were inversely correlated ( $r = -0.65$ ) among 61 individuals on an ambient diet. Several years later, Burgess et al<sup>21</sup> reported a similar association in 10 hypertensives ( $r = -0.76$ ). Interestingly, in a randomized trial that documented a 14-mm Hg decrease in SBP with vitamin D supplementation compared with placebo, the authors also noted a trend toward a decrease in circulating angiotensin II (Ang II) levels ( $-13.1$  pg/mL;  $P = 0.14$ ) relative to placebo.<sup>22</sup>

To test the hypothesis that there is a mechanistic role for vitamin D in the regulation of the RAS in humans, we examined the relation between plasma 25(OH)D concentration with both circulating renin and Ang II levels, as well as the renal plasma flow (RPF) response to infused Ang II, which correlates inversely with endogenous intrarenal RAS activity,<sup>23-27</sup> among 184 normotensive individuals.

### Methods

#### Study Population

Participants in this study included 184 normotensive white and black men and women, recruited as healthy volunteers from the general population, who completed RPF studies in high sodium balance at 1 of 4 General Clinical Research Centers, including Brigham and Women's Hospital in Boston; the University of Utah Medical Center in Salt Lake City, Utah; Vanderbilt University Hospital in Nashville, Tennessee; and the Hôpital Européen Georges Pompidou in Paris, France. We examined normotensive participants because of our previous observations that 25(OH)D levels are inversely associated with the risk of incident hypertension among previously normotensive individuals.<sup>3,4</sup> We analyzed participants during high sodium balance because the range of RPF responsiveness in high sodium balance is greater than in low sodium balance and, thus, allows for easier detection of interindividual differences.<sup>24</sup> In addition, high sodium balance more closely mimics the average ambient diet and, thus, enhances generalizability of the study findings. The institutional review boards at each of the contributing institutions approved the study, and all of the participants provided written informed consent.

Participants were classified as normotensive, defined by a seated blood pressure  $< 140/90$  mm Hg, not taking antihypertensive medications, and, furthermore, not having a first-degree relative with hypertension onset before the age of 60 years. All of the participants underwent a screening history and physical and laboratory examinations. Other exclusion criteria included diabetes mellitus, chronic kidney disease (defined as a serum creatinine  $> 1.5$  mg/dL), or other significant medical problem, including coronary heart disease or active malignancy.

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## Study Protocol

All of the participants consumed a high-salt diet (200 mmol of sodium) for 3 to 7 days before the study. High sodium balance was defined by a 24-hour urine sodium excretion  $\geq 150$  mmol. Participants were admitted to the General Clinical Research Center the night before the RPF study.

On the day of the study, 2 intravenous catheters were inserted, one for infusions and the other for blood collection. Participants remained supine during the study. An 8-mg/kg loading dose of para-aminohippuric acid (PAH) was administered 60 minutes before the administration of Ang II. This loading dose was immediately followed by a continuous infusion of PAH at 12 mg/min to achieve plasma PAH concentrations in the middle of the range at which tubular secretion dominates excretion. At this concentration of PAH, clearance is independent of plasma levels, and effective RPF (as PAH clearance) was calculated from steady-state plasma PAH concentrations, as described previously.<sup>28,29</sup> Effective RPF was normalized to a body surface area of 1.73 m<sup>2</sup>. Ang II was then infused at 3 ng/kg per minute for 55 minutes. Three pre-Ang II measurements of RPF and 3 post-Ang II measurements of RPF were made.

## Day of Study Measurements

Blood pressure was monitored every 5 minutes during the study using a Dinamap automated device (Critikon). All of the PAH assays were performed by the same technician using an autoanalyzer technique. The intra-assay and interassay coefficients of variation for the PAH assay were  $<5\%$  and  $<10\%$ , respectively. PRA was measured using a radioimmunoassay (DiaSorin). The sensitivity of this assay is 0.01 ng/mL per hour, and the coefficient of variation is  $<10\%$ . The Ang II radioimmunoassay (ALPCO) measures Ang II by a double-antibody radioimmunoassay on plasma samples that are pretreated with a "homemade" mixture of enzyme inhibitors against aminopeptidase, protease, and Ang-converting enzyme. The sensitivity of this method is 0.6 pg/mL (range: 0.6 to 500.0 pg/mL), and the coefficient of variation is  $<15\%$ . Urine sodium was measured by an ion-selective electrode using automatically diluted specimens (ISE Indirect) and the COBAS Integra 400 (Roche Diagnostics).

## Measurement of 25(OH)D

Measurements of 25(OH)D in plasma were performed using a radioimmunoassay from the DiaSorin corporation. The sensitivity of this assay is 4 ng/mL, and the coefficient of variation ranges from 4.4% to 8.4%.

Before measuring 25(OH)D on all of the study participants, we performed a pilot study to determine whether assaying 25(OH)D levels on stored frozen samples was feasible and reliable. We thawed, aliquoted, and measured 25(OH)D levels on frozen samples from 19 participants who also had 25(OH)D levels measured on fresh samples from the original day of the high sodium study. The correlation coefficient comparing levels from fresh and frozen samples was 0.97.

## Statistical Analyses

Participants were categorized by vitamin D status on the basis of 25(OH)D levels: optimal ( $\geq 30.0$  ng/mL), insufficient (15.0 to 29.9 ng/mL), and deficient ( $<15.0$  ng/mL). Baseline characteristics according to vitamin D status were analyzed by univariate linear regression (for continuous variables) or the  $\chi^2$  test of trend (for categorical variables). For the baseline association between vitamin D status and both PRA and Ang II concentration, we also performed multivariable linear regression adjusting for age, race, and body mass index (BMI, calculated as the weight in kilograms divided by the height in meters squared).

The primary outcome, specifically RPF response to Ang II infusion, was calculated by subtracting the median post-Ang II PAH clearance from the median baseline pre-Ang II PAH clearance. At the present time, there is no readily available means of directly assessing intrarenal RAS activity in humans. The RPF response to infused Ang II is an indirect measure of the intrinsic activity of the

**Table. Baseline Study Characteristics Stratified by Level of Plasma 25(OH)D**

Characteristic	$\geq 30$ ng/mL (N=37)	15.0 to 29.9 ng/mL (N=108)	$<15$ ng/mL (N=39)	P
Age, y	42.2 (9.5)	40.0 (12.2)	38.2 (13.5)	0.15
Black, %	0	5	55	$<0.001$
Women, %	59	50	51	0.49
BMI, kg/m <sup>2</sup>	25.8 (4.2)	25.3 (3.7)	25.1 (4.3)	0.41
Systolic blood pressure, mm Hg	111 (12)	110 (12)	114 (10)	0.21
Diastolic blood pressure, mm Hg	66 (8)	66 (8)	71 (6)	0.006
24-h urine sodium, mmol	241 (87)	217 (78)	225 (79)	0.43
Basal RPF, mL/min per 1.73 m <sup>2</sup>	575 (125)	590 (143)	572 (97)	0.99

Continuous variables are expressed as mean (SD); categorical variables are expressed as percentage. P values were determined by univariate linear regression (for continuous variables) or the  $\chi^2$  test (for categorical variables).

intrarenal RAS,<sup>27</sup> and numerous lines of evidence indicate that the RPF response to Ang II in high sodium balance is inversely correlated with endogenous RAS activity.<sup>23–26</sup>

The association between vitamin D status and the primary outcome of RPF response to Ang II was analyzed using multivariable linear regression, with participants who had optimal vitamin D status defined as the reference group. Unadjusted analyses were first performed to generate data for an intuitively interpretable figure, and then multivariable analyses were performed to adjust these estimates for age, race, sex, BMI, systolic blood pressure, diastolic blood pressure, 24-hour urine sodium, and baseline (pre-Ang II) RPF. The relations between vitamin D category with PRA and Ang II were also analyzed with linear regression. Because black race was strongly associated with lower 25(OH)D levels, we performed a secondary analysis after excluding black individuals to determine whether our findings were being driven exclusively by race.

As exploratory analyses, we also examined the association between vitamin D status and baseline levels of aldosterone and also the blood pressure response to infusion of Ang II. Finally, we explored whether there was an interaction between vitamin D status and sex for our primary end point.

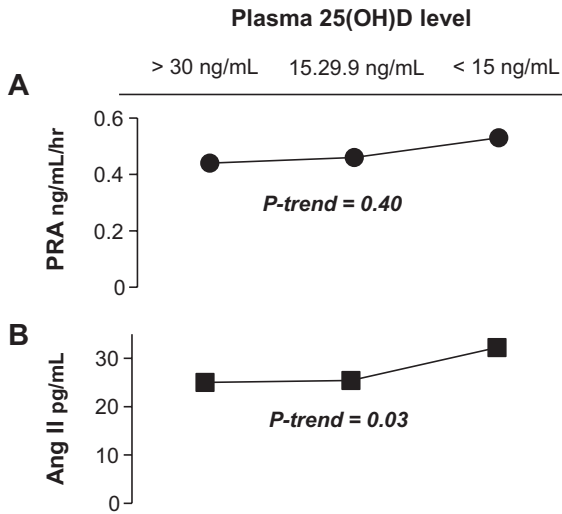
Values in the results are mean  $\pm$  SE, unless otherwise specified. A P value  $<0.05$  was considered statistically significant. P values were 2-tailed. All of the statistical analyses were performed using SAS statistical software, version 9.1.

## Results

### Baseline Characteristics

The mean age of participants was 40.1 years (SD: 12.0 years); 52.2% were women, and 14.7% were black. Plasma 25(OH)D levels ranged from 4.8 to 52.4 ng/mL (mean: 22.3 ng/mL), and 79.9% of participants had levels that were insufficient or deficient defined as a level  $<30.0$  ng/mL. The mean BMI of the group was 25.3 kg/m<sup>2</sup> (SD: 3.9 kg/m<sup>2</sup>), and the mean systolic/diastolic blood pressures were 111/67 mm Hg (SD: 12/8 mm Hg). Sodium loading was successful as confirmed by 24-hour urine collection (mean sodium: 224 mmol; SD: 80 mmol).

Baseline characteristics according to category of 25(OH)D are displayed in the Table. Individuals with lower 25(OH)D levels were more likely to be black ( $P<0.001$ ) and had higher



**Figure 1.** Association between vitamin D status and PRA and Ang II levels in high sodium balance. A, PRA. B, Ang II concentration. Mean values are shown. Results are from univariate linear regression.

diastolic blood pressures ( $P=0.006$ ). Other baseline characteristics did not significantly vary according to vitamin D status.

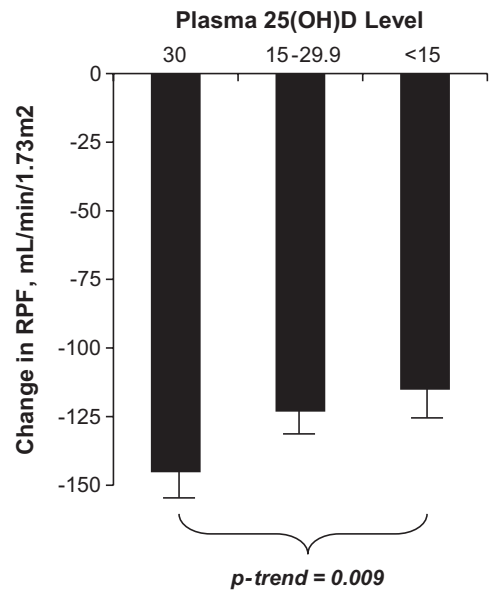
**Vitamin D and Baseline PRA and Ang II**

PRA did not significantly differ according to vitamin D status (Figure 1A). PRA levels were low, as anticipated in high sodium balance. Among all of the participants, mean PRA was  $0.09 \pm 0.10$  ng/mL per hour higher among vitamin D-deficient individuals compared with those with sufficient vitamin D levels ( $P$  trend=0.40). In multivariable models adjusted for age, race, and BMI, PRA was  $0.17 \pm 0.13$  ng/mL per hour higher among those with vitamin D deficiency ( $P$  for trend=0.17). When the analysis was restricted to whites, the mean PRA was  $0.15 \pm 0.14$  ng/mL per hour higher comparing vitamin D-deficient to -sufficient individuals ( $P$  for trend=0.24). Circulating aldosterone levels also did not significantly differ between categories of 25(OH)D ( $P$  for trend=0.37).

In contrast, Ang II was significantly higher among individuals with lower 25(OH)D levels (Figure 1B). Comparing individuals with vitamin D deficiency with those whose 25(OH)D levels were  $\geq 30$  ng/mL, mean Ang II levels were  $7.2 \pm 3.6$  pg/mL higher ( $P$  for trend=0.04) in univariate analyses. After adjustment for age, race, and BMI, Ang II levels were  $9.9 \pm 4.4$  pg/mL higher among vitamin D-deficient individuals ( $P$  for trend=0.01). When this analysis was restricted to white individuals, this same comparison documented  $10.8 \pm 4.6$  pg/mL higher mean Ang II levels among vitamin D-deficient individuals ( $P$  for trend=0.005).

**Vitamin D and RPF Response to Ang II Infusion**

RPF responses to Ang II infusion in high sodium balance according to vitamin D status are shown in Figure 2. The mean unadjusted decline in RPF with Ang II infusion in participants whose 25(OH)D level was  $\geq 30$  ng/mL was  $145 \pm 13$  mL/min per  $1.73 \text{ m}^2$ . The mean decline in RPF



**Figure 2.** Association between vitamin D status and Ang II-induced changes in RPF while in high sodium balance. Values are means, and error bars represent SEs. Results are from univariate linear regression.

among individuals whose 25(OH)D level was  $< 15$  ng/mL was comparatively blunted ( $115 \pm 10$  mL/min per  $1.73 \text{ m}^2$ ;  $P$  for trend=0.001). To account for potential confounding, we used multivariable linear regression adjusting for age, BMI, basal RPF, and race, and the association remained statistically significant; the response to Ang II infusion among vitamin D-deficient individuals was  $45 \pm 13$  mL/min per  $1.73 \text{ m}^2$  less than among those with optimal 25(OH)D levels ( $P$  for trend=0.009). Additional adjustment for the study center had no impact on the results. The change in systolic blood pressure in response to Ang II infusion was also less (by 2 mm Hg) among those with vitamin D deficiency compared with those with optimal levels, but this was not statistically significant ( $P$  for trend=0.47).

To determine whether the association was present in a racially uniform population, we reanalyzed the data after excluding black individuals. The RPF response to Ang II remained significantly blunted among individuals with lower plasma 25(OH)D levels after multivariable adjustment ( $P$  for trend=0.02). Too few blacks were included to analyze them in isolation. We did not observe any interaction between 25(OH)D levels and sex regarding the RPF response to Ang II infusion ( $P$  for interaction=0.43).

**Discussion**

Although animal models strongly suggest that vitamin D suppresses the RAS, human data are largely lacking. To our knowledge, this is the first human study to examine the association between plasma 25(OH)D levels and control of the RAS under rigorously controlled dietary conditions. We found that, among normotensive individuals, lower 25(OH)D levels were associated with higher circulating Ang II levels and a blunted RPF response to exogenous Ang II infusion, both findings consistent with activation of the RAS in the setting of lower plasma 25(OH)D.

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Observational studies strongly support an inverse association between plasma 25(OH)D levels and blood pressure and hypertension.<sup>1–14</sup> In addition to numerous cross-sectional analyses, 2 prospective studies have demonstrated that lower baseline 25(OH)D levels are associated with an increased risk of incident hypertension.<sup>3,4</sup> In the first, which included 613 men and 1198 women who did not have hypertension at baseline, those with 25(OH)D levels <15 ng/mL (vitamin D deficiency) compared with  $\geq 30$  ng/mL had a relative risk for incident hypertension of 2.7 after adjusting for multiple demographic and lifestyle factors.<sup>4</sup> The second prospective study also considered levels of parathyroid hormone plus numerous other biomarkers as potential confounders and found that individuals with vitamin D insufficiency (25-hydroxyvitamin D level <30 ng/mL) had a 1.5-fold higher risk of developing hypertension compared with those with optimal levels.<sup>3</sup>

Several putative mechanisms for this relation have been forwarded, including associations between vitamin D deficiency and endothelial dysfunction,<sup>22,30–33</sup> inflammation,<sup>34–37</sup> and insulin resistance.<sup>38–41</sup> However, a major proposed mechanism was documented by Li et al<sup>15</sup> in mice lacking the vitamin D receptor gene. Absence of vitamin D signaling in these animals led to an increase in renin gene expression and circulating Ang II levels. When placed on a high-salt diet, these knockout mice had slight reductions of renin and Ang II but maintained levels substantially higher than wild-type mice on a similar diet.<sup>15</sup> Furthermore, renin levels remained elevated despite normalization of plasma calcium concentrations, and injection of 1,25(OH)<sub>2</sub>D reduced renin expression in wild-type mice.<sup>15,16</sup> Other animal models support these findings, demonstrating that mice lacking the 1 $\alpha$ -hydroxylase gene have a similar phenotype<sup>19</sup> and that 1,25(OH)<sub>2</sub>D analogs help suppress Ang II-mediated kidney injury in diabetic and in 5/6 nephrectomized rats.<sup>17,18</sup>

In contrast, few human studies have examined this relationship. The first human study to investigate the association examined 10 normotensive individuals, as well as 51 hypertensive individuals, on ambient diets divided into low-renin, normal-renin, and high-renin status.<sup>20</sup> The authors reported the highest levels of 1,25(OH)<sub>2</sub>D in low-renin hypertensives compared with normal- and high-renin hypertensives ( $P < 0.01$  for both comparisons), as well as normotensives ( $P < 0.01$ ). Among all 61 individuals, there was an inverse correlation between PRA and 1,25(OH)<sub>2</sub>D ( $r = -0.65$ ;  $P < 0.001$ ).<sup>20</sup> The second human study included 10 high-renin hypertensive individuals who were studied initially on a 5-day low-sodium diet (10 mmol/d) and then again after a 5-day high-sodium diet (100 mmol/d).<sup>21</sup> The authors found that changing from a low-sodium to high-sodium diet led to significant increases in urine calcium excretion and 1,25(OH)<sub>2</sub>D levels, plus a decrease in PRA. The changes in 1,25(OH)<sub>2</sub>D concentration and PRA were inversely correlated ( $r = -0.76$ ;  $P = 0.01$ ).<sup>21</sup> The authors hypothesized that sodium loading led to an increase in calcium excretion, which, in turn, led to an increase in 1,25(OH)<sub>2</sub>D levels, which then suppressed PRA by increasing juxtaglomerular cell calcium concentrations.<sup>21</sup> Levels of 25(OH)D were not measured in either of these studies. One randomized trial of vitamin D supplementation

(with ergocalciferol) documented effects on markers of the RAS.<sup>22</sup> Sugden et al<sup>22</sup> randomized 34 individuals with type 2 diabetes mellitus and 25(OH)D levels <20 ng/mL to receive either 100 000 IU of vitamin D<sub>2</sub> or placebo. Along with a 6-ng/mL increase in 25(OH)D level at 8 weeks of follow-up with supplementation, systolic blood pressure declined by 14 mm Hg compared with placebo. In addition, Ang II levels decreased by 13.1 pg/mL in the vitamin D group relative to the change in the placebo group; conversely, active renin levels (not PRA) were increased in the vitamin D group relative to the change in the placebo group by 2.6 ng/mL.<sup>22</sup> Neither of these results was statistically significant.

We found that, among 184 normotensive individuals in high sodium balance, lower plasma 25(OH)D levels were associated with significantly higher circulating Ang II concentrations, as well as a blunted RPF response to infused Ang II. Both of these findings support the concept that vitamin D deficiency may be associated with upregulation of the RAS. In contrast, although PRA was higher among individuals with vitamin D insufficiency and deficiency, the association was not statistically significant. This may, in part, be because of insufficient statistical power. Suppression of PRA in the setting of sodium loading may have resulted in levels and ranges that were too low and too narrow to detect a statistical difference given the sample size. It is possible that an association may have been detected had the sample been considerably larger. On the other hand, it is also possible that, unlike animals, vitamin D in humans may influence tissue-level production of renin rather than systemic levels. Alternatively, our findings may reflect a renin-independent mechanism for vitamin D suppression of systemic and local Ang II. Indeed, vitamin D signaling may inhibit the expression of angiotensinogen by inhibition of nuclear factor  $\kappa$ B transcription factors,<sup>42,43</sup> and angiotensinogen may be converted by cathepsins to Ang II in the absence of renin.<sup>44,45</sup>

Our study examined plasma 25(OH)D levels; the aforementioned animal and previous human investigations of vitamin D and the RAS, in contrast, focused on levels of 1,25(OH)<sub>2</sub>D. Because plasma 25(OH)D is not under homeostatic control, whereas 1,25(OH)<sub>2</sub>D levels are homeostatically regulated, individuals with low 25(OH)D levels may have normal levels of 1,25(OH)<sub>2</sub>D; however, the 2 hormones are generally well correlated.<sup>46</sup> In addition, and more importantly, the epidemiological data supporting a relation between vitamin D and hypertension, as well as vitamin D and cardiovascular disease, are essentially limited to analyses of 25(OH)D and not 1,25(OH)<sub>2</sub>D. To invoke the RAS as a mechanism to explain the epidemiological data, therefore, it was critical to analyze levels of 25(OH)D.

Finally, several lines of evidence suggest that 25(OH)D, like 1,25(OH)<sub>2</sub>D, may be an “active” hormone. The 1 $\alpha$ -hydroxylase gene is widely expressed,<sup>47</sup> so 25(OH)D may be converted to 1,25(OH)<sub>2</sub>D locally in various tissues, bypassing the need for conversion in the proximal tubule and thereby having autocrine and paracrine effects. Furthermore, depending on the conformation of the vitamin D receptor (*cis* or *trans*), it may be located either in the cytoplasm or at the plasma membrane, the latter localization associated with its ability to activate second messengers, such as protein



kinase-C, mitogen-activated protein kinase, and phosphatidylinositol 3-kinase.<sup>48</sup> New data suggest that, whereas the affinity of cytoplasmic vitamin D receptor is greatest for 1,25(OH)<sub>2</sub>D, the binding affinity of 25(OH)D for membrane-associated vitamin D receptor matches that of 1,25(OH)<sub>2</sub>D.<sup>49</sup> Considering that 25(OH)D concentrations are 1000-fold higher than those of 1,25(OH)<sub>2</sub>D, it is increasingly apparent that 25(OH)D may be an important active circulating hormone.

### Limitations

Our study has limitations. First and foremost, this analysis was cross-sectional, and, therefore, we cannot demonstrate directionality of the association, nor can we comment on causality. It is possible that upregulation of the RAS somehow reduced plasma 25(OH)D levels through an unknown mechanism. Although we adjusted for relevant confounders, such as BMI, race, and basal RPF, it is possible that our findings represent residual confounding. Only a randomized, controlled intervention could address these possibilities. Second, we did not measure plasma levels of parathyroid hormone or 1,25(OH)<sub>2</sub>D, and, therefore, cannot examine whether these hormones mediate or confound our observed association. Third, we had insufficient power to examine these associations in black individuals; this is important because both vitamin D deficiency and hypertension are more prevalent in blacks than in whites. Fourth, we did not gather dietary information nor did we know whether participants were using vitamin D supplements. Nonetheless, this study was cross-sectional, and plasma 25(OH)D levels were measured on blood samples that were collected within one week of the day that the study was performed. Therefore, dietary or supplemental vitamin D intake was reflected in the plasma 25(OH)D levels analyzed. Because we lacked information about calcium intake, however, we were unable to investigate interactions between vitamin D status and dietary and supplemental calcium. Fifth, we did not have any measurement of renal prostaglandin production in our participants or whether they used nonsteroidal anti-inflammatory drugs. Because renal prostaglandins may regulate the 1 $\alpha$ -hydroxylase enzyme and, thus, influence conversion of 25(OH)D to 1,25(OH)<sub>2</sub>D,<sup>50</sup> it would have been interesting to perform an additional analysis to test an interaction between vitamin D and renal prostaglandins.

### Perspectives

Our findings in normotensive individuals are consistent with an association between low plasma 25(OH)D levels and upregulation of the RAS. These findings may partly explain the higher risk of developing hypertension observed among individuals with vitamin D insufficiency and deficiency. Randomized trials should be performed to confirm or refute these observations.

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### Disclosures

None.

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