


# A randomized intervention study to evaluate the effect of calcitriol therapy on the renin-angiotensin system in diabetes

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## Abstract

**Background:** Prior studies suggest that vitamin D therapy may decrease cardiovascular disease risk in type 2 diabetes (T2DM) by lowering renin-angiotensin system (RAS) activity. However, randomized human intervention studies to evaluate the effect of vitamin D receptor (VDR) agonists on RAS activity are lacking.

**Objective:** The objective of this article is to investigate the effect of direct VDR activation with calcitriol on circulating RAS activity and vascular hemodynamics in T2DM.

**Methods:** A randomized, double-blinded, and placebo-controlled study wherein 18 participants with well-controlled T2DM without chronic kidney disease (CKD) were administered calcitriol or placebo for three weeks was conducted. Outcome measures included plasma renin activity (PRA), serum and urinary aldosterone, mean arterial pressure (MAP) before and after an infusion of angiotensin II, and renal plasma flow (RPF) via *para*-aminohippurate clearance.

**Results:** Despite an increase in 1,25(OH)<sub>2</sub>D with calcitriol administration (45.4 to 61.8 pg/ml,  $p = 0.03$ ) and no change with placebo, there were no significant differences in PRA, serum or urinary aldosterone, baseline and angiotensin II-stimulated MAP, or basal and angiotensin II-stimulated RPF between interventions.

**Conclusion:** In this randomized and placebo-controlled study in participants with T2DM without CKD, calcitriol therapy to raise 1,25(OH)<sub>2</sub>D levels, when compared with placebo, did not significantly change circulating RAS activity or vascular hemodynamics.

## Keywords

1,25-dihydroxyvitamin D, calcitriol, vitamin D, renin-angiotensin system, type 2 diabetes mellitus

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## Introduction

Excessive renin-angiotensin system (RAS) activity and low vitamin D levels have both been linked to the development of cardiovascular disease and diabetic nephropathy.<sup>1</sup> Low 25-hydroxyvitamin D (25(OH)D) levels have been associated with higher RAS activity in humans,<sup>2,3</sup> which has been postulated as one potential reason by which vitamin D deficiency associates with cardiovascular and kidney disease.<sup>4–6</sup> The circulating and local renal tissue RAS mediates renal-vascular physiology and disease<sup>7,8</sup> and RAS inhibitors are known to mitigate the development of nephropathy.<sup>9</sup> Since higher circulating RAS activity has been observed in states of vitamin D deficiency, it has been hypothesized that treatment with vitamin D may reduce RAS activity and mitigate the development of renal-vascular disease, similar to RAS inhibitors.<sup>10</sup>

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Animal studies have shown that direct vitamin D receptor (VDR) activation with 1,25-dihydroxyvitamin D (1,25(OH)<sub>2</sub>D) suppresses renin expression<sup>11</sup> and lowers RAS activity. In mouse models of type 2 diabetes mellitus (T2DM), both VDR agonists and RAS inhibitors blunt the development of diabetic nephropathy, and in combination, act synergistically to prevent diabetic nephropathy by downregulating the RAS.<sup>12,13</sup> VDR and 1 $\alpha$ -hydroxylase functional knockout mice both show over-stimulation of the RAS and high blood pressure that can be abrogated by RAS inhibitors or calcitriol.<sup>14–16</sup>

In humans, prior intervention studies focusing on hypertensives with vitamin D deficiency have demonstrated mixed results. In one large randomized and blinded study, vitamin D<sub>3</sub> therapy lowered circulating aldosterone.<sup>17</sup> However, in another small non-randomized study, neither calcitriol nor vitamin D<sub>3</sub> therapy influenced RAS activity in hypertensives with vitamin D deficiency.<sup>18</sup> Our own prior single-arm and open-label physiology study in obese hypertensive individuals demonstrated that high-dose cholecalciferol supplementation to increase 25(OH) D and 1,25(OH)<sub>2</sub>D resulted in improvement of renal-vascular hemodynamics in a manner similar to the effect of angiotensin-converting enzyme (ACE) inhibitors.<sup>10</sup> This finding extended prior observational studies suggesting that lower vitamin D was associated with higher renal-vascular tissue RAS activity.<sup>3</sup> However, the limitations of this study were that it was not randomized, did not include placebo control, and did not assess renal-vascular hemodynamic parameters in diabetes, for which the risk for nephropathy is greater.

Based on robust animal data and suggestive human data, we hypothesized that direct VDR activation with calcitriol (i.e. 1,25(OH)<sub>2</sub>D) could lower RAS activity and improve vascular hemodynamics in human diabetes. Herein, we performed a double-blinded, randomized controlled trial in participants with T2DM to evaluate the impact of calcitriol on the circulating RAS and renal-vascular hemodynamics.

## Materials and methods

### Study participants

Participants between the ages of 18 and 70 were recruited from the local community in Boston, MA, USA. A study physician assessed eligibility for the study during a screening evaluation that included a detailed history, physical examination, and laboratory assessment (Visit 1). Participants were enrolled if they met inclusion criteria, which included having T2DM with hemoglobin (Hb)A1c < 8.5% on diet, oral hypoglycemic agents, incretin analogs, or a single basal insulin injection. Additional inclusion criteria included normal blood pressure or stage 1 hypertension on 0–1 antihypertensive medications, and normal complete blood count, liver function testing, urinalysis, and

electrocardiogram. Participants were excluded if they had: chronic kidney disease (defined as estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m<sup>2</sup>); poorly controlled T2DM (defined as HbA1c  $\geq$ 8.5% or use of >1 daily insulin injection); known diabetic microvascular complications (prior documented retinopathy, nephropathy, or peripheral neuropathy requiring a medical intervention); stage 2–3 hypertension or the use of >1 antihypertensive medication; history of kidney stones, parathyroid or granulomatous disorders, liver failure, congestive heart failure, coronary artery disease, or cerebrovascular accident; or active use of tobacco or recreational drugs.

All participants provided informed consent, and the study protocol was approved by the institutional review board at the Brigham and Women's Hospital (NCT01635062).

### Medication washout protocol

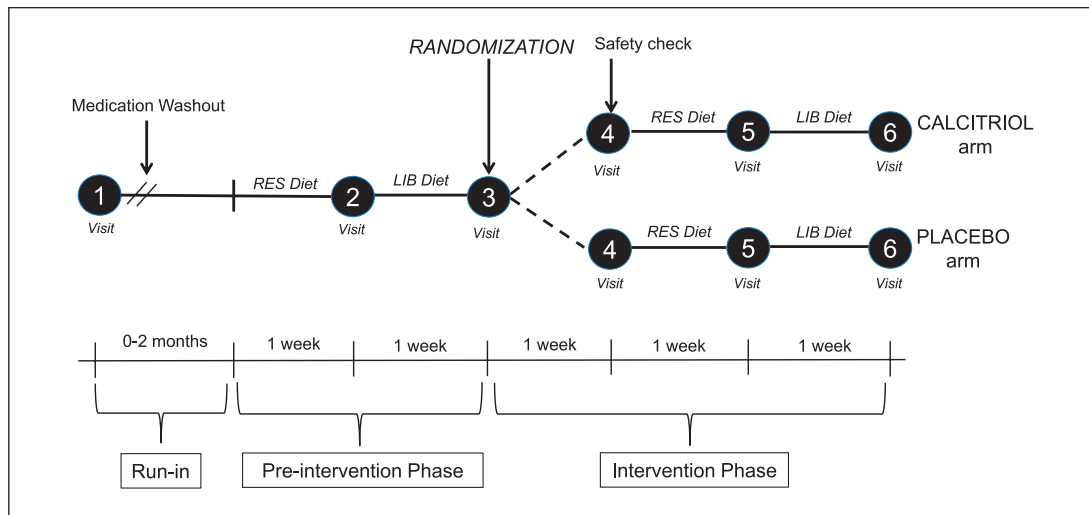
Participants underwent an antihypertensive medication washout, if applicable, prior to randomization to eliminate their confounding influence on the RAS. The safety protocol for this drug washout has been previously described.<sup>10</sup> Of the major antihypertensive medication classes, RAS inhibitors were withdrawn for two months, beta-blockers and diuretics were withdrawn for one month, and calcium-channel blockers were withdrawn for two weeks prior to the initiation of study procedures; this washout was maintained throughout the entirety of the study. During this washout period and during the study, participants were monitored with a home sphygmomanometer, reporting their daily measurements to study staff weekly to ensure safety while off their antihypertensive regimen. If home blood pressures exceeded 159/99 mmHg, participants were withdrawn from the study for safety and resumed on their antihypertensive medication regimen.

### Dietary manipulation to assess the RAS

All study visits were conducted under controlled dietary conditions to minimize confounding and variability of the RAS and calcium physiology. Prior to each study visit, participants were prescribed a study diet for one week with fixed amounts of sodium, potassium, and calcium, as previously described.<sup>19</sup> All diets contained 100 mEq/d of potassium and 600 mg/d of calcium carbonate. Sodium content was either restricted (RES) to maximally stimulate the RAS (10 mmol/d) or liberalized (LIB) to maximally suppress the RAS (200 mmol/d). All diets were prepared by professional dietary staff in our institutional Clinical Research Center (CRC).

### Study visits

The primary aim was to assess the effect of calcitriol on RAS activity, with secondary aims including effect on renal plasma flow (RPF) and RPF response to angiotensin



**Figure 1.** Study schema.

RES: restricted sodium diet; LIB: liberal sodium diet.

II (AngII). Study visits were performed in the CRC at Brigham and Women's Hospital to control posture, time of day, and time of medication administration. Detailed profiling of the RAS was performed at baseline and after randomization to calcitriol or placebo to assess the impact of calcitriol on RAS activity (Figure 1).

Following the screening visit (Visit 1) to establish eligibility and enrollment, participants returned for Visit 2 after completing the RES diet. The objective of Visit 2 was to assess the baseline (pre-randomization) maximally stimulated circulating RAS (plasma renin activity (PRA) and aldosterone). Blood sampling was performed at 9 a.m. after 60 minutes of supine posture. Participants also submitted a 24-hour urine collection, which was used to assess the aldosterone excretion rate (AER), sodium, calcium, and creatinine. Blood pressure measurements were recorded at 10-minute intervals throughout the visit.

Following Visit 2, participants completed the LIB diet and returned one week later for Visit 3, for which they were admitted to the CRC and remained in supine posture overnight. The objective of Visit 3 was to assess the baseline (pre-intervention) maximally suppressed circulating RAS (PRA and aldosterone) and hemodynamic parameters. At 8 a.m. the following morning, after 12 hours of supine rest, PRA and serum aldosterone were measured at baseline and again after a 90-minute infusion of AngII (at a rate of 1 ng/kg/min), as previously described.<sup>10</sup> The objective of post-AngII measurements was to assess the adrenal aldosterone and vascular responses to AngII. Further, RPF was measured using *para*-aminohippurate clearance as previously described.<sup>10</sup> RPF was measured at baseline and following an infusion of AngII. Mean arterial pressure (MAP) was measured every five minutes throughout the study using a GE Dinamap Pro Monitor. The baseline and post-AngII MAP were defined as the average of five consecutive readings taken two minutes apart.

Following Visit 3, participants were randomized to either calcitriol or placebo (see below), returned for a safety visit (Visit 4—see description below) one week later, and then returned for post-intervention measurements. Visit 5 was identical to Visit 2: Participants completed the RES diet and returned to assess whether the maximally stimulated RAS was influenced following two weeks of randomized intervention. Visit 6 was identical to Visit 3: Participants completed the LIB diet and returned to assess whether vascular hemodynamics and the RAS were influenced following three weeks of randomized intervention (Figure 1).

### Calcitriol therapy protocol

Following study Visit 3, participants were randomized by the research pharmacy to receive calcitriol or placebo using two-block, double-blind randomization. Participants were initially instructed to take one pill (one pill = 0.25  $\mu$ g of calcitriol or placebo) twice daily, for a total daily dose of 0.5  $\mu$ g of calcitriol, or placebo. Participants returned for a safety visit (Visit 4) one week later at which serum calcium, phosphate, and creatinine were checked (Figure 1). If all safety labs were within the normal range, the blinded medication was increased by one pill daily to two pills in the morning and one pill at night (0.75  $\mu$ g of calcitriol or placebo daily).

### Laboratory measurements

Parathyroid hormone (PTH) (Beckman Coulter, Fullerton, CA), 25OHD (Diasorin Inc, Stillwater, MS), 1,25OH<sub>2</sub>D (Diasorin), and serum and urinary electrolytes (including calcium and phosphate) were measured at each study visit. PRA (Diasorin, Stillwater, MN) and aldosterone (Siemens, Los Angeles, CA) were measured as described above. Twenty-four-hour urinary aldosterone excretion was

measured at all study visits. RPF was measured using *para*-aminohippurate (PAH) clearance as previously described.<sup>10</sup> PAH was measured in triplicate at baseline and averaged to assess basal RPF, and again in triplicate following the AngII infusion to assess the renal-vascular response to AngII.

### Statistical analyses

Mean (standard deviation, SD) values are reported, except PRA and aldosterone, which are reported as median (interquartile range, IQR) owing to non-normal distribution. Paired *t*-tests were used to evaluate the impact of each intervention (calcitriol or placebo) on circulating RAS and hemodynamic parameters. Two-way analysis of variance (ANOVA) with interaction was used to compare the effect of each intervention on outcomes of interest. For nonparametric outcome variables (i.e. PRA and aldosterone), the Kruskal-Wallis test was used for paired data, and non-parametric outcomes were log-transformed in two-way ANOVA interaction analysis. A two-tailed *p* value of <0.05 was considered statistically significant.

Data analysis was performed using SAS (version 9.4) statistical software (SAS Institute, Cary, NC).

## Results

### Study participants

A total of 41 participants were enrolled and gave informed consent to participate. After initial consent to participate, only 18 of the 41 participants maintained eligibility criteria, retained interest in participation, and were able to complete the antihypertensive medication washout protocol and dietary phases required for baseline Visits 2 and 3 (Supplemental Figure 1). Randomized participants (*n* = 18) were well matched based on screening characteristics (Table 1). The mean age of participants was 50.8 (SD 7.6), with 11 out of 18 (61%) male, and 13 out of 18 (72%) Caucasian (all others were of African descent). All participants had well-controlled T2DM as assessed by HbA1c (mean HbA1c 6.9 ± 0.8%). The mean 25OHD concentration was 31.8 ng/dl (SD 19.7 ng/dl) in the calcitriol group and 28.7 ng/dl (SD 14.8 ng/dl) in the placebo group.

### Change in vitamin D and mineral metabolites with the randomized intervention

Following three weeks of randomized intervention therapy, 1,25OH<sub>2</sub>D levels increased significantly with calcitriol use (pre-intervention Visit 3: 45.4 ± 18.2 pg/ml, post-intervention Visit 6: 61.8 ± 11.2 pg/ml, *p* = 0.03), but did not change in those taking placebo (pre-intervention Visit 3: 40.3 ± 21.3 pg/ml, post-intervention Visit 6: 45.0 ± 7.9 pg/ml, *p* = 0.54). There were no significant changes in serum calcium, urinary calcium, phosphate, or PTH

during this randomized intervention (Table 2), and no participants required dose reduction of calcitriol or placebo at the safety visit. There were no significant adverse events (including hypercalcemia, hyperphosphatemia, and hypotension).

### The impact of calcitriol therapy on maximally stimulated RAS activity

Following two weeks of randomized intervention therapy, calcitriol did not significantly change maximally stimulated PRA (pre-intervention Visit 2: 2.3 (IQR 1.5, 4.5) ng/ml\*min, post-intervention Visit 5: 1.7 (IQR 0.7, 3.2) ng/ml\*min, *p* = 0.54) (Table 3). Maximally stimulated aldosterone, as well as maximally stimulated 24-hour urinary aldosterone excretion, did not change with calcitriol intervention (Table 3).

### The impact of calcitriol therapy on maximally suppressed RAS activity and basal vascular hemodynamics

Following three weeks of randomized intervention therapy (comparison of Visit 3 and Visit 6 data on LIB diet), there were no changes in maximally suppressed RAS activity, or basal MAP and RPF (Table 4).

### The impact of calcitriol therapy on vascular sensitivity to AngII

As expected, MAP increased and RPF decreased in response to AngII both in the placebo and calcitriol groups. However, the magnitude and percentage change in MAP and RPF with AngII infusion did not differ before and after calcitriol intervention. Pre-calcitriol MAP increased by 9.3 ± 6.0 mmHg in response to AngII, and post-calcitriol MAP increased by 10.4 ± 6.9 mmHg in response to AngII, *p* = 0.72. Pre-calcitriol RPF decreased by 89.2 ± 80.5 cc/min/1.73 m<sup>2</sup> in response to AngII, while post-calcitriol RPF decreased by 94.5 ± 66.8 cc/min/1.73 m<sup>2</sup> in response to AngII, *p* = 0.89. These differences were no different from those observed with placebo.

## Discussion

In participants with well-controlled diabetes, we found that calcitriol, a potent VDR agonist, did not lower RAS activity or significantly alter hemodynamic parameters such as blood pressure and RPF. Further, the systemic and renal vascular response to an infusion of exogenous AngII was not modified by calcitriol intervention. Though the sample size of our study was small, our study design went to great lengths to ensure that confounders of the RAS were eliminated (posture, dietary sodium, antihypertensive medications) to permit confidence in inter- and intra-individual comparisons. Further, the study



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

	Placebo N = 9	Calcitriol N = 9	p value
<b>Age, years</b>	50.7 (5.0)	51.0 (10.4)	0.93
<b>Female, number (%)</b>	5 (55)	2 (22)	0.33
<b>White, number (%)</b>	5 (55)	8 (88)	0.29
<b>BMI, kg/m<sup>2</sup></b>	31.4 (6.1)	31.5 (4.7)	0.97
<b>On anti-diabetic therapy, number (%)</b>	9 (100)	7 (77)	0.47
Metformin	7 (78)	6 (67)	0.60
Sulfonylurea	3 (33)	3 (33)	1.0
GLP-1 agonist	1 (11)	2 (22)	0.53
DPP4-inhibitor	1 (11)	1 (11)	1.0
$\alpha$ -Glucosidase inhibitor	0	1 (11)	0.30
Insulin	3 (33)	2 (22)	0.60
<b>Duration of diabetes (years)</b>	6.0 (4.7)	7.2 (4.1)	0.60
<b>Systolic blood pressure, mmHg</b>	125.3 (18.3)	124.4 (14.9)	0.91
<b>Diastolic blood pressure, mmHg</b>	80.3 (13.6)	79.7 (7.4)	0.91
<b>MAP, mmHg</b>	95.3 (14.4)	94.6 (9.4)	0.90
<b>Pulse, bpm</b>	72 (12.1)	76.4 (9.7)	0.40
<b>HbA1c, %</b>	6.8 (0.9)	7.0 (0.6)	0.70
<b>eGFR, ml/min/1.73 m<sup>2</sup></b>	102.3 (17.4)	99.8 (12.5)	0.73
<b>Serum creatinine, mg/dl</b>	0.81 (0.1)	0.82 (0.1)	0.83
<b>Serum potassium, mmol/l</b>	4.4 (0.3)	4.4 (0.4)	0.78
<b>25(OH)D, ng/ml</b>	31.8 (19.7)	28.7 (14.8)	0.72
<b>PTH, pg/ml</b>	22.5 (12.2)	25.1 (7.5)	0.71

Values are mean (SD) unless otherwise specified.

BMI: body mass index; bpm: beats per minute; GLP-1: glucagon-like peptide-1; DPP4: dipeptidyl peptidase-4; MAP: mean arterial pressure; HbA1c: hemoglobin A1c; eGFR: estimated glomerular filtration rate; 25(OH)D: 25-hydroxyvitamin D; PTH: parathyroid hormone.

**Table 2.** Change in vitamin D and mineral metabolites on LIB diet.

	Calcitriol group, N = 9			Placebo group, N = 9			p ANOVA
	Pre-intervention	Post-intervention	p value	Pre-intervention	Post-intervention	p value	
<b>1,25(OH)<sub>2</sub>D, pg/ml</b>	45.4 (18.2)	61.8 (11.2)	0.03	40.3 (21.3)	45.0 (7.9)	0.54	0.27
<b>PTH, pg/ml</b>	36.6 (11.8)	30.5 (12.4)	0.30	33.9 (12.6)	32.5 (12.7)	0.82	0.57
<b>Serum calcium, mg/dl</b>	8.89 (0.26)	8.92 (0.16)	0.75	8.91 (0.42)	8.84 (0.46)	0.75	0.67
<b>24-hour urinary calcium, mg/24 hours</b>	254.9 (125.6)	332.2 (140.7)	0.24	207.5 (83.3)	237.1 (132.2)	0.58	0.56
<b>Serum phosphate, mg/dl</b>	3.4 (0.3)	3.5 (0.5)	0.72	4.2 (0.5)	4.2 (0.5)	0.55	0.51

Values are mean (SD), unless otherwise noted.

ANOVA: analysis of variance; LIB: liberal sodium diet; 1,25(OH)<sub>2</sub>D: 1,25-dihydroxyvitamin D; PTH: parathyroid hormone.

design included modulations of dietary sodium to maximally suppress and stimulate the RAS to permit assessments across the entire dynamic range of RAS activity before and after randomized intervention. Since calcitriol administration to raise 1,25(OH)<sub>2</sub>D levels is considered to be a supra-physiologic maneuver to activate the VDR, one that bypasses the physiologic 1 $\alpha$ -hydroxylation regulated by PTH, the current results argue against a clinically relevant RAS-lowering effect of direct VDR activation in human diabetes that has been robustly observed in animal studies.<sup>11,15,16,20</sup>

Our current study is best interpreted in comparison to prior intervention studies examining the influence of vitamin D on the RAS. In a post-hoc analysis of a randomized,

controlled, and double-blinded study of 188 participants with vitamin D deficiency and hypertension randomized to vitamin D<sub>3</sub> (2800 IU daily) or placebo for eight weeks, Gröbler et al. observed a modest but significant decrease in plasma aldosterone levels with vitamin D<sub>3</sub> therapy;<sup>17</sup> however, there were no changes in renin, and the study design and outcome assessments did not include protocols to physiologically control confounders of the RAS. In contrast, in a separate and smaller open-label study that used fixed dietary sodium control, Bernini et al. showed that neither calcitriol therapy ( $n = 10$ ; 0.5 mcg daily for one week) nor vitamin D<sub>3</sub> therapy ( $n = 18$ ; single 300,000 IU dose over eight weeks) influenced renin activity or

**Table 3.** Change in maximally stimulated circulating RAS parameters with intervention (calcitriol vs. placebo) on RES diet.

	Calcitriol group, N = 9			Placebo group, N = 9			p ANOVA
	Pre-intervention	Post-intervention	p value	Pre-intervention	Post-intervention	p value	
<b>PRA, ng/ml*hour</b>	2.3 (1.5, 4.5)	1.7 (0.7, 3.2)	0.54	4.6 (3.0, 5.3)	2.6 (2.4, 3.1)	0.17	0.83
<b>Aldosterone, ng/dl</b>	21.9 (17.6, 36.7)	19.9 (16.6, 32.7)	0.57	19.2 (13.8, 23.9)	16.0 (10.1, 21.9)	0.51	0.81
<b>24-hour urinary AER, µg/d</b>	37.3 (17.0)	36.8 (15.1)	0.96	32.1 (19.8)	36.0 (19.0)	0.71	0.74

Values are mean (SD) except PRA and aldosterone, which is median (interquartile range (IQR)).

P values are t-test, except for PRA and aldosterone, which is Kruskal-Wallis test (nonparametric).

P ANOVA for PRA and aldosterone uses log-transformed values.

ANOVA: analysis of variance; RAS: renin-angiotensin system; RES: restricted sodium diet; PRA: plasma renin activity; AER: aldosterone excretion rate.

**Table 4.** Change in circulating RAS parameters and baseline vascular hemodynamics with interventions (calcitriol vs. placebo) on LIB diet.

	Calcitriol group, N = 9			Placebo group, N = 9			p ANOVA
	Pre-intervention	Post-intervention	p value	Pre-intervention	Post-intervention	p value	
<b>PRA, ng/ml*hour</b>	0.3 (0.25, 0.35)	0.25 (0.1, 0.45)	0.59	0.15 (0.1, 0.4)	0.2 (0.1, 0.55)	0.74	0.53
<b>Aldosterone, ng/dl</b>	2.0 (2.0, 3.7)	2.9 (2.0, 7.6)	0.36	2.0 (2.0, 2.6)	2.0 (2.0, 2.7)	0.87	0.51
<b>24-hour urinary AER, µg/d</b>	9.5 (8.5)	10.3 (9.9)	0.86	13.5 (19.6)	13.8 (21.8)	0.98	0.96
<b>MAP, mmHg</b>	98.6 (7.2)	97.2 (7.6)	0.69	91.1 (11.8)	94.1 (10.9)	0.58	0.49
<b>RPF, cc/min/1.73 m<sup>2</sup></b>	650.7 (203.5)	634.3 (154.1)	0.86	608.9 (155.2)	608.3 (128.6)	0.58	0.89

Values are mean (SD) except PRA and aldosterone, which is median (interquartile range (IQR)).

P values are t-test, except for PRA and aldosterone, which is Kruskal-Wallis test (nonparametric).

P ANOVA for PRA and aldosterone uses log-transformed values.

ANOVA: analysis of variance; RAS: renin-angiotensin system; LIB: liberal sodium diet; PRA: plasma renin activity; AER: aldosterone excretion rate, MAP: mean arterial pressure; RPF: renal plasma flow.

circulating AngII and aldosterone in hypertensives with vitamin D deficiency.<sup>18</sup> In this regard, our current study: (1) focused on individuals with diabetes in whom animal data suggest the most profound vitamin D-RAS interaction,<sup>20</sup> (2) used higher-dose and longer duration of calcitriol to ensure robust and supra-physiologic VDR agonism, (3) controlled for numerous confounders of the RAS to increase confidence in measurements, and (4) assessed vascular parameters that are known to be influenced by RAS activity. However, despite these efforts, we observed no effect of calcitriol on RAS or vascular parameters.

Our prior open-label study of high-dose cholecalciferol supplementation in non-diabetic obese individuals with hypertension ( $n = 14$ ; 15,000 IU of vitamin D<sub>3</sub> daily for one month) showed compelling evidence of a renal-vascular tissue RAS-lowering effect,<sup>10</sup> which is counter to our current findings, even though both studies produced significant increases in 1,25OH<sub>2</sub>D levels with intervention. There were several differences between these study designs that might account for some of this disparity. Participants in our prior study had vitamin D deficiency (mean 25OHD of 16.6 ng/ml) at baseline, whereas in our current study most individuals were 25OHD sufficient (mean 25OHD 28.7 ng/ml at baseline). It is possible that those with underlying vitamin D deficiency have higher RAS activation at baseline and therefore are more readily

modifiable. In addition, the studies used differing patient populations (obese in the former, diabetes in the current study), study designs with differing risks for bias (open-label and single-arm in the former, double-blinded and placebo-controlled with randomization in the current), and duration of vitamin D intervention (four weeks in the former and two to three weeks in the current). Given these differences, it is possible that obese and non-diabetic individuals with vitamin D deficiency may preferentially benefit from VDR activation.

Animal models have proposed a robust effect of vitamin D on the RAS. Li et al. showed that VDR knockout mice had significantly higher renin and AngII expression,<sup>16</sup> and other studies in VDR knockout mouse models have demonstrated higher RAS activity and increased renin messenger RNA expression in cardiac tissues.<sup>14</sup> In addition, Li et al. demonstrated that 1,25OH<sub>2</sub>D administration lowered renin levels in wild-type mice,<sup>16</sup> consistent with more recent findings of reductions in renin and angiotensinogen after treatment with calcitriol in nephrectomized rats.<sup>21</sup> In mice lacking the ability to synthesize 1,25OH<sub>2</sub>D, RAS activity was upregulated but could be suppressed with the administration of 1,25OH<sub>2</sub>D.<sup>15</sup> It has been proposed that these effects may be mediated by downregulation of *renin* gene transcription; in a cell culture model,

administration of 1,25OH<sub>2</sub>D suppressed *renin* gene transcription<sup>11</sup> in juxtaglomerular cells.

In human observational studies, vitamin D deficiency (25OHD level <15 ng/ml) and insufficiency (25OHD level 15.0-29.9 ng/ml) have been associated with higher circulating AngII levels compared to vitamin D-sufficient individuals (25OHD level ≥30 ng/ml),<sup>3</sup> and 25OHD levels have been inversely associated with renin activity.<sup>2</sup> Furthermore, the combination of 25(OH)D levels and functional VDR polymorphisms synergistically predict renin activity.<sup>22</sup>

Given compelling animal and observation data, numerous intervention studies investigating the effect of vitamin D therapy on RAS activity and endothelial function emerged. A majority of these studies were performed using the widely used, inactive versions of vitamin D: cholecalciferol and ergocalciferol. Several studies have demonstrated benefits of short-term cholecalciferol in lowering renin,<sup>23,24</sup> improving endothelial function,<sup>25</sup> and improving tissue sensitivity to AngII.<sup>10</sup> However, an increasing number of randomized trials have resulted in negative conclusions regarding RAS-mediated benefits of cholecalciferol therapy. In a trial in 84 normotensive, vitamin D-deficient, overweight participants, McMullan et al. showed that eight weeks of ergocalciferol 50,000 IU per week had no effect on PRA or AngII levels, 24-hour blood pressure, or the RPF response to captopril when compared to placebo.<sup>26</sup> Other studies have similarly shown no effect of cholecalciferol on RAS activity, as previously discussed,<sup>1,8</sup> nor on blood pressure,<sup>27-30</sup> arterial stiffness,<sup>27,31</sup> or endothelial function.<sup>27</sup> Given the discordant results of these trials, larger and more long-term randomized controlled trials may help determine if there is benefit of vitamin D supplementation on cardiovascular outcomes, with some currently underway.<sup>32</sup>

### Strengths and limitations

Our study was a randomized, double-blinded, placebo-controlled trial with well-matched study arms. We used the activated form of vitamin D, calcitriol, to increase the potency of VDR activation above prior studies that have relied on the inactive pro-hormone, cholecalciferol. The study protocol was performed with rigorous adherence to sodium- and calcium-controlled study diets, as well as careful control of posture and timing of data collection, in a clinically relevant study population (T2DM).

Limitations of our study include the short duration of intervention and the small sample size, which may have limited the power to detect certain changes. For example, the lack of a statistically significant decline in PTH with calcitriol intervention may have been a result of insufficient power. However, since pharmacologic RAS inhibitors, such as ACE inhibitors, would be expected to induce marked changes to the RAS and vascular hemodynamics in this sample size and study duration, the fact that we did not

observe any effect with calcitriol is notable and supports a lack of a clinically meaningful effect. In addition, we included participants with varying degrees of baseline vitamin D status; therefore, we cannot exclude that VDR-mediated RAS suppression may be observable in those with more pronounced vitamin D deficiency. Further, it is well known that there are racial and ethnic differences in RAS activity and vitamin D physiology;<sup>33,34</sup> however, our study design and sample size did not permit subanalyses to further explore these potentially important factors. Lastly, we measured surrogate markers of clinical disease, and this, combined with our small sample size and short study duration, make our findings difficult to extrapolate to conclusions regarding clinical outcomes, such as cardiovascular health.

In conclusion, in participants with diabetes who were relatively vitamin D sufficient, potent VDR activation with calcitriol did not modify RAS activity or alter renal-vascular function under strictly controlled settings. Although several studies continue to evaluate the influence of vitamin D therapy in mitigating cardiovascular risk, the current findings argue against a clinically meaningful vitamin D-RAS interaction.

### Declaration of conflicting interests


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