Effect of calcitriol treatment on arterial stiffness in people with type 2 diabetes and stage 3 chronic kidney disease

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Funding information
Diabetes UK. Grant/Award Number: 09/0003886

Aims: Active vitamin D deficiency is associated with increased aortic-pulse wave velocity (Ao-PWV) in people with type 2 diabetes (T2DM) and chronic kidney disease (CKD). There are no randomised controlled trials investigating the effect of active vitamin D treatment on Ao-PWV in people with T2DM and CKD.

Methods: A 48-week duration single-centre randomised double-blind parallel-group trial examined the impact of oral 1,25 dihydroxyvitamin D (calcitriol 0.25 mcg OD) as compared to placebo on a primary endpoint of Ao-PWV. People with T2DM and stable stage 3 CKD with intact parathyroid hormone (iPTH) level >30 pg/mL were eligible.

Results: In total, 127 (70% male) people were randomised (calcitriol n = 64 or placebo n = 63). There was no change in Ao-PWV observed, mean ± standard deviation (SD), in the calcitriol group of 11.79 (±2.5) to 12.08 (±3.0) m/s as compared to 10.90 (±2.4) to 11.39 (±2.6) m/s with placebo. The between-treatment group adjusted mean (95% confidence interval [CI]) change was 0.23 (−0.58 to 1.05) m/s, P = .57. No effect of calcitriol was observed on central arterial pressures, albuminuria, serum calcium or phosphate levels. However, iPTH fell with calcitriol treatment (mean [95% CI] between-group difference of −27.8 (−42.3 to −13.2) pg/mL, P < .001.

Conclusion: In T2DM and stage 3 CKD, calcitriol as compared to placebo does not improve Ao-PWV or other markers of arterial stiffness. Our study does not provide evidence for the use of active vitamin D for improving arterial stiffness in T2DM with stage 3 CKD.

KEYWORDS
arterial stiffness, CKD, type 2 diabetes, vitamin D
1 | BACKGROUND

Between 20% and 40% of people with type 2 diabetes (T2DM) have stage 3 chronic kidney disease (CKD) (eGFR 30–59 mL/min). Cardiovascular disease (CVD) is the main cause of death in people with T2DM and stage 3 CKD. This enhanced risk is not fully explained by traditional cardiovascular risk factors, such as age, hypertension, dyslipidaemia and smoking. Recent studies implicate active vitamin D (1,25-dihydroxyvitamin D or 1,25(OH)2D [calcitriol]) deficiency as a potential modifiable risk factor for CVD and renal disease prevention. Activation of vitamin D receptor in the vascular system may result in reduction in arterial stiffness and vascular calcification in diabetic animals. People with T2DM have accelerated vascular ageing of the aorta with resultant increased aortic pulse wave velocity (Ao-PWV). Reversibility of Ao-PWV is an important modifiable risk factor for CVD and mortality in patients with CKD and increased Ao-PWV is also independently associated with deterioration in renal function. There are conflicting data on the impact of active vitamin D treatment on cardio-renal biomarkers and vascular indices in people with and without CKD. Most studies are small, of short duration of treatment (<24 weeks) and enrolled people with multiple CKD aetiologies. Furthermore, the majority of these studies included people with advanced kidney disease such as stage 4 and 5 CKD. There are no randomised controlled studies on the effects of active vitamin D on Ao-PWV in people with diabetes and stage 3 CKD.

The aim of this proof-of-concept trial was to study the effect of active vitamin D (calcitriol) supplementation as add-on treatment to existing medications on the primary endpoint of Ao-PWV in people with T2DM and stage 3 CKD.

2 | METHODS

This was a proof-of-concept, single-centre 48 week prospective randomised double-blind parallel-group placebo-controlled intervention trial (EudraCT number 2010-018285-23).

2.1 | Patients

We included people with T2DM aged between 40 and 75 years (inclusive) with stable CKD stage 3 (eGFR 30–59 mL/min) on the two most recent consecutive measurements (within 12 months) with a clinical diagnosis of diabetic kidney disease and history of an elevated albuminuria (urine albumin to creatinine ratio ≥2.5 mg/mmol in men and ≥3 mg/mmol in women or timed urine albumin excretion rate [AER] > 20 mcg/min) or elevated urine protein to creatinine ratio. Other inclusion criteria were normal corrected serum calcium (2.1–2.6 mmol/L), normal phosphate (0.8–1.5 mmol/L), and an intact parathyroid hormone (iPTH) between 30 pg/mL and 200 pg/mL at screening visit or in the 3 months preceding screening visit. Exclusion criteria included an iPTH ≥ 200 pg/mL at study entry, history of non-diabetic or obstructive kidney disease, presence of connective tissue diseases known to affect arterial vasculature or atrial fibrillation or other cardiac rhythm disorders (as these will impact on Ao-PWV measurement), pregnancy, history of cardiovascular or cerebrovascular event in the preceding 6 months, or treatment with inactive or active vitamin D treatment. The study duration was from 2013 to 2018. The trial was conducted in compliance with the principles of the Declaration of Helsinki (1996), and in accordance with all applicable regulatory requirements. The study was approved by Guy’s Research Ethics Committee. All participants provided informed consent and were recruited from a university hospital diabetes renal clinic. The study was funded by a research grant from Diabetes UK.

2.1.1 | Interventions

Participants were randomised in a 1:1 ratio to either the calcitriol or placebo treatment by means of a computer-generated random sequence. The active vitamin D was prescribed as a fixed dose of 0.25 mcg once daily (OD). It was estimated that a daily calcitriol dose of 0.25 mcg would translate to a change in Ao-PWV of between 1.5 and 2 m/s. This dose was also chosen in view of the population studies in stage 3 and 4 CKD that suggested a dose of 0.25 mcg daily was associated with reduction in mortality and that potential impact on hypercalcaemic events was low in similar studies in stage
The study medication was manufactured and supplied by an accredited university hospital pharmacy manufacturing unit as per national and international quality control regulations and requirements.

2.1.2 | Endpoint definitions

The primary endpoint of the trial was change from baseline in Ao-PWV after 48 weeks' treatment with calcitriol as compared to placebo.

All measurements and procedures were performed with the participants in the fasted state and having refrained from nicotine, alcohol and caffeine for at least the previous 10 hours. Brachial blood pressure was measured in triplicate in the supine position by an automated sphygmomanometer (Omron Digital Blood Pressure Monitor HEM907, Bannockburn, IL). Ao-PWV was determined from carotid and femoral pressure waveforms obtained noninvasively by applanation tonometry (Millar tonometer, Millar Instruments, Houston, TX) using the Sphygmocor system (Atcor, Sydney, Australia) as previously described. Central blood pressure determinations including the aortic augmentation index (Aix) were also measured using the same methods.

Secondary endpoints included urine AER, augmentation index, brachial and central systolic and diastolic blood pressure, estimated GFR (by modification of diet in renal disease formula), serum calcium and phosphate levels. Urine AER was calculated from the median of three nonconsecutive timed overnight urine specimens collected 1 week before Ao-PWV measurement. Blood samples for serial serum creatinine, serum calcium and phosphate levels for safety purposes were measured in an accredited hospital laboratory. Levels of 25(OH)D3, and 1,25(OH)2D were analysed using enzyme linked immunosassay, intact FGF-23 by enzyme linked immunosassay and iPTH by chemiluminescence immunoassay at an accredited central laboratory.

3 | STATISTICAL METHODS

As this was a proof-of-concept study, it was estimated that 60 participants per treatment group would provide 90% power with a 0.05 target alpha (two-sided) to detect a difference in Ao-PWV between calcitriol and placebo of 0.9 m/s. Descriptive statistics were used for the analysis of demographic and clinical features. Data were compared using an unpaired t-test (for continuously normally distributed variables), Mann–Whitney test (for continuous variables not normally distributed) and \( \chi^2 \) test (for categorical variables). Variables were tested for normality by Shapiro test and Q-Q plots and further on mean, standard deviation and 95% confidence intervals were calculated for the normally distributed variables and median and interquartile range for the non-normally distributed variables. The change in Ao-PWV was analysed using an analysis of covariance (ANCOVA). The least squares mean, 95% confidence interval and P-values for treatment difference are displayed. ANCOVA analysis was performed for all normally distributed variables. Variables that were not normally distributed were log transformed for analyses. The primary population used was the intention-to-treat population. The endpoint was defined as the last available post-randomisation measurement of Ao-PWV. All statistical analysis was performed within R studio (version 1.3.1073) under R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

4 | RESULTS

A total of 140 participants were randomised to placebo (n = 68) or calcitriol (n = 72). Of the 140 participants randomised, 127 (placebo n = 64 and calcitriol n = 63) were eligible for analyses. Figure 1 describes the participant flow. Table 1 describes the baseline characteristics of this cohort. The majority of participants were male (70%), with a median (interquartile range) age of 67 (60.5–70) years. We had an ethnically diverse cohort that reflected our local population with 45% Caucasian ethnicity, 36% of African-Caribbean, 16% of Asian and 2% of other ethnicities, respectively. Mean duration of diabetes was 19.6 years and 72% were on insulin treatment. Metformin was used in 52% of the cohort. More than 85% of participants were on lipid-lowering treatment with statins or fibrates. Use of renin angiotensin system (RAS) inhibitors was frequent with 90% of participants on treatment. No participants were on sodium-glucose co-transporter-2 (SGLT-2) inhibitors and only 15% participants were on glucagon-like peptide-1 (GLP-1) receptor agonists. There was no significant difference between groups in the use of RAS inhibitors or other medications.

All participants met the inclusion criterion iPTH > 30 pg/mL with median (interquartile range) of 73.95 (54.8, 105) pg/mL at baseline with no difference between treatment groups at baseline. Of note 1,25(OH)2D and 25(OH)D vitamin D levels at baseline was not an entry criterion and all levels were only measured after completion of the study. The level of 1,25(OH)2D was 26.4 (20.7–33.1) pg/mL and 41.5 (30.5–54.9) ng/mL for 25(OH)D.

Table 2 describes the baseline and end-of-treatment values for the primary endpoint Ao-PWV and selected secondary endpoints in the calcitriol treatment and placebo treatment groups. Between treatment group difference (adjusted and unadjusted for baseline levels) are also shown.

Following 48 weeks’ treatment with calcitriol, there was no significant change in Ao-PWV, mean (± SD) 11.79 (±2.5) to 12.08 (±3.0) m/s as compared to 10.90 (±2.4) to 11.39 (±2.6) m/s with placebo (P > .05 for both) (Figure 2). The between treatment group mean adjusted difference (95% confidence interval) in Ao-PWV was 0.23 (−0.58 to1.05) m/s, P = .57 (Figure 2).

As demonstrated in Table 2 we did not observe any significant effect of calcitriol on other indices of arterial stiffness, such as augmentation index, or central aortic blood pressures. Similarly, no between treatment group effect of calcitriol was observed on urinary AER or eGFR during the trial.
No significant changes in 25(OH)D and 1,25(OH)2D levels were observed during treatment with calcitriol, which has also been noted in other studies. This may be related to calcitriol acting as a strong inducer of CYP24A1, an enzyme that metabolises/inactivates both 25(OH)D and 1,25(OH)2D. However, as expected, we did observe a significant reduction in iPTH levels with calcitriol treatment from baseline, median (interquartile range), of 77.5 (56.7 to 111.4) to end of treatment 60.9 (41.0 to 88.10) pg/mL, a median fall of 18.3 (20.7 to 15.9) pg/mL, P < .009. In contrast, in the placebo group, iPTH levels rose modestly during the 48 weeks from baseline 60.9 (41.0 to 88.10) to 86.4 (62.9 to 115.3) pg/mL, P = .09 at end of treatment. We observed a significant between treatment group difference reduction in iPTH with calcitriol with a mean (95% confidence interval) difference of 27.8 (42.3 to 13.2) pg/mL, P < .001. FGF-23 levels also increased significantly with calcitriol treatment from 77.74 (60.74 to 96.54) to 103.28 (81.1 to 143.16) with a between treatment group difference (adjusted for baseline) of 30.6 (14.8 to 46.3) pg/mL, P < .001. There were, however, no significant between treatment group differences in serum calcium or phosphate levels.

In further post-hoc analyses, where we evaluated the per protocol cohort of people who completed all study visits, we did not observe any significant impact of treatment with calcitriol on primary or secondary endpoints (data not shown).

During the trial there were two deaths, one in the calcitriol and one in the placebo groups. Overall, calcitriol was well tolerated with no treatment-related serious adverse effects (SAE) related to hypercalcaemia or other known adverse effects of calcitriol reported. A summary of all adverse and serious adverse events is reported in Tables S1–S3 in the Supporting Information.

**5 | DISCUSSION**

We observed that in people with T2DM and stage 3 CKD, treatment with calcitriol as compared to placebo does not improve Ao-PWV, albuminuria or indices of central arterial blood pressure. Reduced levels of vitamin D (both inactive and active metabolites) has been proposed as a modifiable risk factor to reduce the burden of CVD in people with CKD. The vitamin D receptor is found in the heart, ...
vascular wall and the kidney and its activation regulates several genes apart from those involved in calcium and phosphate metabolism.\textsuperscript{5} Vitamin D deficiency is also implicated in vascular dysfunction, and in vitro and animal data demonstrate that treatment with vitamin D analogues, such as calcitriol, can reduce arterial stiffness by multiple direct and indirect mechanisms including inhibition of mineralisation in vascular smooth muscle cells, anti-inflammatory and antioxidative effects.\textsuperscript{5,6}

However, most of the data that support this hypothesis are from observational studies with their inherent limitations, and there is limited evidence from randomised controlled trials.\textsuperscript{5,19,20,25} Ao-PWV, the gold standard measure of arterial stiffness, is an independent predictor of CVD outcomes, mortality and progression of kidney disease.\textsuperscript{9} Several studies have demonstrated inverse associations between vitamin D levels and measures of arterial stiffness including Ao-PWV.\textsuperscript{26–28} However, there is modest and often conflicting evidence regarding the impact of vitamin D supplementation or replacement on arterial stiffness reported in recent studies and meta-analyses.\textsuperscript{13,29–31} People with diabetes and CKD are at enhanced risk of CVD and progression of kidney dysfunction and there is

\begin{table}
\centering
\caption{Baseline characteristics of 127 people with type 2 diabetes and stage 3 CKD randomised to calcitriol or placebo treatment}
\begin{tabular}{|l|c|c|}
\hline
 & All participants (n = 127) & Calcitriol (n = 63) & Placebo (n = 64) \\
\hline
Gender male & 89 (70.1%) & 41 (65.1%) & 48 (75.0%) \\
Age, years* & 67 (60.5 to 70) & 67 (60.5 to 69.5) & 67 (60.8 to 70) \\
Ethnicity & & & \\
Caucasian & 58 (45.7%) & 26 (41.3%) & 32 (50.0%) \\
African-Caribbean & 46 (36.2%) & 24 (38.1%) & 22 (34.4%) \\
Asian & 20 (15.7%) & 12 (19.0%) & 8 (12.5%) \\
Other & 3 (2.4%) & 1 (1.6%) & 2 (3.1%) \\
BMI, kg/m² & 32.5 (6.1) & 32.6 (6.3) & 32.4 (6) \\
Weight, kg & 94.4 (20.1) & 95.1 (22.1) & 93.8 (18.1) \\
SBP, mmHg & 146.4 (19.8) & 149.7 (21.9) & 143.1 (17) \\
DBP, mmHg & 76.6 (11) & 77.3 (11) & 76 (11) \\
MAP, mmHg & 99.9 (12.6) & 101.4 (13.2) & 98.4 (11.9) \\
PP, mmHg & 11.3 (2.5) & 11.8 (2.5) & 10.9 (2.5) \\
Augmentation index, % & 22.5 (8.5) & 23.4 (9.1) & 21.7 (7.8) \\
Central SBP mmHg & 131.2 (20.2) & 134.5 (22.9) & 128.0 (17.0) \\
Central DBP mmHg & 76.4 (12.1) & 76 (13.5) & 77 (10.5) \\
Central PP mmHg & 56.1 (18.6) & 60.1 (21.7) & 52.1 (14.8) \\
Urine albumin excretion rate mcg/min* & 45.1 (11.5 to 171.7) & 53.4 (10.8 to 132.6) & 23.6 (11.9 to 230.2) \\
Serum creatinine, μmol/L & 157.9 (32.7) & 147.9 (35.6) & 148.3 (36.7) \\
eGFR ml/min per 1.73 m² & 41.0 (9.8) & 40.7 (10.6) & 41.3 (9.2) \\
HbA1c, % & 8.1 (1.4) & 8.4 (1.4) & 7.8 (1.3) \\
Vitamin 1,25(OH)2D, pmol/L* & 26.3 (20.6 to 32.8) & 26.5 (18.8 to 31.2) & 26.0 (21.1 to 33.2) \\
Vitamin 25(OH)D, nmol/L* & 41.5 (30.5 to 54.9) & 42.4 (30.3 to 55.1) & 39.9 (30.8 to 52.5) \\
Serum calcium, mmol/L & 2.35 (0.1) & 2.36 (0.1) & 2.34 (0.1) \\
Serum phosphate, mmol/L & 1.07 (0.16) & 1.09 (0.14) & 1.09 (0.18) \\
iPTH, pg/mL* & 74.0 (54.8 to 105.0) & 77.5 (56.7 to 111.2) & 69 (52.7 to 99.0) \\
FGF-23, pg/mL* & 73.3 (60.5 to 96.2) & 77.8 (60.7 to 96.5) & 72.0 (60.5 to 95.1) \\
Haemoglobin, g/L & 12.1 (1.6) & 12.1 (1.6) & 12.21 (1.6) \\
Serum albumin, g/L & 43.4 (3.4) & 43.3 (3.4) & 43.5 (3.4) \\
Total cholesterol, mmol/L & 4.0 (1.0) & 4.1 (1.1) & 4.0 (0.9) \\
HDL cholesterol, mmol/L & 1.3 (0.4) & 1.3 (0.4) & 1.3 (0.3) \\
LDL cholesterol, mmol/L & 1.9 (0.9) & 1.9 (0.9) & 2.0 (0.8) \\
\hline
\end{tabular}
\footnotesize{Data mean (standard deviation) or median * interquartile range shown. Abbreviations Ao-PWV: aortic pulse wave velocity; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure; BMI: body mass index; FGF-23: fibroblast growth factor 23; HDL: high density lipoprotein; LDL: low density lipoprotein.}
|                      | Calcitriol versus placebo | Placebo \( (n = 64) \) | Calcitriol \( (n = 63) \) |
|----------------------|---------------------------|--------------------------|--------------------------|
| **Ao-PWV, m/s**      |                           |                          |                          |
| Baseline             | 10.9 (2.4)                | 11.8 (2.5)               |
| End of treatment     | 11.4 (2.6)                | 12.1 (3.00)              |
| Unadjusted change \(95\% \text{ CI}\) | \(-0.11 (-0.77, 0.99), P = .80\) |                          |                          |
| **Augmentation index, %** |                           |                          |                          |
| Baseline             | 21.7 (7.8)                | 23.4 (9.1)               |
| End of treatment     | 21.5 (6.7)                | 22.3 (7.4)               |
| Unadjusted change \(95\% \text{ CI}\) | \(-0.92 (-1.5, 3.4), P = .45\) |                          |                          |
| **Central SBP, mmHg** |                           |                          |                          |
| Baseline             | 128.0 (17.0)              | 134.5 (22.9)             |
| End of treatment     | 127.0 (18.1)              | 135.1 (24.6)             |
| Unadjusted change \(95\% \text{ CI}\) | \(-0.09 (-2.06, 1.90), P = .92\) |                          |                          |
| **Central DBP, mmHg** |                           |                          |                          |
| Baseline             | 77.0 (10.5)               | 76.0 (13.5)              |
| End of treatment     | 75.1 (10.1)               | 77.26 (11.6)             |
| Unadjusted change \(95\% \text{ CI}\) | \(-1.8 (-2.9, -0.7), P = .22\) |                          |                          |
| **Central PP, mmHg** |                           |                          |                          |
| Baseline             | 52.1 (14.8)               | 60.1 (21.7)              |
| End of treatment     | 56.5 (40.7)               | 58.1 (19.3)              |
| Unadjusted change \(95\% \text{ CI}\) | \(-3.06 (-6.8, 0.7), P = .11\) |                          |                          |
| **Brachial SBP, mmHg** |                           |                          |                          |
| Baseline             | 143.1 (17.0)              | 149.7 (21.9)             |
| End of treatment     | 142.1 (18.3)              | 149.3 (26.2)             |
| Unadjusted change \(95\% \text{ CI}\) | \(-0.7 (-11.7, 10.4), P = .90\) |                          |                          |
| **Brachial DBP, mmHg** |                           |                          |                          |
| Baseline             | 76.0 (11.0)               | 77.3 (11.0)              |
| End of treatment     | 75.7 (10.0)               | 76.2 (11.3)              |
| Unadjusted change \(95\% \text{ CI}\) | \(-0.74 (-2.52, 4.01), P = .65\) |                          |                          |
| **Brachial MAP, mmHg** |                           |                          |                          |
| Baseline             | 98.4 (11.9)               | 101.4 (13.2)             |
| End of treatment     | 98.7 (12.1)               | 99.8 (14.3)              |
| Unadjusted change \(95\% \text{ CI}\) | \(-0.30 (-3.18, 2.70), P = .86\) |                          |                          |
| **Brachial PP, mmHg** |                           |                          |                          |
| Baseline             | 68.7 (14.6)               | 69.7 (18.40)             |
| End of treatment     | 68.9 (16.9)               | 70.6 (19.5)              |
| Unadjusted change \(95\% \text{ CI}\) | \(-0.6 (-5.7, 6.9), P = .84\) |                          |                          |
experimental data that suggests that vitamin D may be a factor that contributes to this heightened risk. Arterial ageing is accelerated in diabetes and markers of arterial stiffness, including Ao-PWV, are increased in diabetic kidney disease. As far as we are aware there are no randomised clinical trials that have evaluated the impact of calcitriol or equivalent active vitamin D treatment on Ao-PWV in patients with T2DM and CKD.

Most studies that have explored the role of vitamin D treatment (including the use of calcitriol or paracalcitriol or cholecalciferol or equivalent) on arterial stiffness have been in predominantly non-

| TABLE 2 (Continued) | Calcitriol versus placebo | Placebo (n = 64) | Calcitriol (n = 63) |
|-----------------------|---------------------------|------------------|---------------------|
| Adjusted change (95% CI) | 2.5 (–3.7, 8.0), P = .38 |                  |                     |
| Urine albumin excretion rate, mcg/min |                  | 23.6 (11.9, 230.2) | 53.4 (10.8, 132.6) |
| Baseline* |                  | 59.8 (14.8, 314.6) | 49.4 (110.0, 234.9) |
| End of treatment* |                  | 0.4 (–0.2, 0.9), P = .33 | 0 (–0.6, 0.5), P = .92 |
| Unadjusted change (95% CI) | –0.3 (–0.8, 0.2), P = .36** |                  |                     |
| Adjusted change (95% CI) | –0.4 (–0.81, 0.07), P = .09* |                  |                     |
| Corrected calcium, mmol/L |                  | 2.34 (0.1) | 2.36 (0.10) |
| Baseline |                  | 2.37 (0.08) | 2.40 (0.12) |
| End of treatment |                  | 0.02 (–0.08, 0.13), P = .06 | 0.04 (–0.1, 0.16), P = .1 |
| Unadjusted change (95% CI) | 0.01 (–0.04, 0.02), P = .46 |                  |                     |
| Adjusted change (95% CI) | 0.02 (–0.01, 0.05), P = .18 |                  |                     |
| Phosphate, mmol/L |                  | 1.09 (0.18) | 1.09 (0.14) |
| Baseline |                  | 1.08 (0.20) | 1.10 (0.15) |
| End of treatment |                  | –0.02 (–0.17, 0.13), P = .76 | 0.01 (–0.13, 0.14), P = .96 |
| Unadjusted change (95% CI) | 0.02 (–0.08, 0.04), P = .45 |                  |                     |
| Adjusted change (95% CI) | 0.02 (–0.03, 0.08), P = .44 |                  |                     |
| iPTH, pg/mL |                  | 81.9 (37.3) | 91.3 (47.4) |
| Baseline |                  | 94.4 (45.7) | 72.9 (48.2) |
| End of treatment |                  | 12.5 (10.3, 14.8), P = .09 | –18.3 (–20.7, –15.9), P = .009 |
| Unadjusted change (95% CI) | –32.7 (–16.5, –48.9), P < .001 |                  |                     |
| Adjusted change (95% CI) | –27.2 (–42.3, –13.2), P < .001 |                  |                     |
| FGF-23, pg/mL |                  | 82.6 (38.2) | 81.1 (33.8) |
| Baseline |                  | 89.6 (38.3) | 121.4 (58.8) |
| End of treatment |                  | 7 (4.9, 9.1), P = .08 | 40.3 (37.9, 42.7), P = <0.001 |
| Unadjusted change (95% CI) | 30.1 (14.1, 46.2), P = <0.001 |                  |                     |
| Adjusted change (95% CI) | 30.6 (14.9, 46.4), P = <0.001 |                  |                     |
| eGFR mL/min |                  | 41.3 (9.1) | 40.7 (10.5) |
| Baseline |                  | 39.6 (8.4) | 37.1 (11.4) |
| End of treatment |                  | –1.7 (–2.8, –0.7), P = .04 | –3.7 (–4.8, –2.5), P = <0.001 |
| Unadjusted change (95% CI) | –2.56 (–0.69, 4.19), P = .15 |                  |                     |
| Adjusted change (95% CI) | –1.93 (–4.25, 0.38), P = .10 |                  |                     |
| HbA1c, % |                  | 7.8 (1.3) | 8.4 (1.4) |
| Baseline |                  | 8.2 (1.8) | 8.4 (1.6) |
| End of treatment |                  | 0.44 (0.01, 0.87), P = .05 | 0.06 (–0.37, 0.49), P = .78 |
| Unadjusted change (95% CI) | –0.3 (–0.09, 0.82), P = .11 |                  |                     |
| Adjusted change (95% CI) | –0.3 (–0.70, 0.20), P = .26 |                  |                     |

Data mean (standard deviation) or median * interquartile range shown ** log data shown as transformed for analyses.
Adjusted change – analyses adjusted for baseline value, mean and 95% confidence intervals shown for between-group differences.
Abbreviations Ao-PWV: aortic pulse wave velocity; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure; BMI: body mass index; FGF-23: fibroblast growth factor 23; HDL: high density lipoprotein; LDL: low density lipoprotein.
These consequences of untreated secondary hyperparathyroidism in CKD include vascular calcifications and arterial stiffness.\textsuperscript{14,15} In our trial treatment with calcitriol was for 48 weeks, which is considerably longer than many other trials.\textsuperscript{7} Our results are consistent with recent meta-analysis in people with CKD (predominantly non-diabetic causes) where there was no conclusive evidence of an effect of vitamin D replacement on indices of arterial stiffness.\textsuperscript{13,30,42}

Epidemiological cross-sectional studies demonstrate that in CKD stage 3 rises in iPTH precede any changes in calcium and phosphate levels. Further, a greater percentage of people with low 1,25 OH\textsubscript{2}D\textsubscript{3} levels had high iPTH, irrespective of 25(OH)D\textsubscript{3} levels.\textsuperscript{43} These changes, which are consistent and physiologically expected, suggest that iPTH may be a reliable and practical early marker of vitamin D status in CKD. At the time of design of our study, the Kidney Disease Outcomes Quality Initiative guidelines recommend iPTH testing in CKD patients at eGFR levels below 60 mL/min/1.73 m\textsuperscript{2}, hence the use of this as an inclusion criterion.\textsuperscript{14} However, we appreciate that many current national guidelines do not recommend routine measurement of iPTH or vitamin D levels in CKD stage 3.\textsuperscript{75}

The limitations of our study are that we used only one form of vitamin D replacement, calcitriol, rather than cholecalciferol or equivalent vitamin D replacement. This was based on the context and information available at the time of study design that cholecalciferol may not always be the most effective replacement in CKD and the supporting observational data from large cohorts of people with stage 3 CKD, which demonstrated an association between calcitriol treatment and improved clinical outcomes including mortality.\textsuperscript{5,19,20} As this was a proof-of-concept trial, our study may be underpowered to detect the changes we estimated which were extrapolated data from observational studies.\textsuperscript{17,21} However, our results are a useful guide for researchers in this area to power future clinical trials.

We only studied one dose of calcitriol of 0.25 mcg daily on the basis of the available observational data at the time of study design, which suggested this was a pragmatic dose to evaluate in a proof-of-concept study such as ours.\textsuperscript{19,20} Of note, a higher dose of calcitriol 0.5 mcg once a day has been studied and failed to demonstrate any significant effect on Ao-PWV.\textsuperscript{14} Another limitation of our study was that only 30% of the study cohort were women.

SGLT-2 inhibitors and GLP-1 receptor agonists have been demonstrated to improve cardio-renal outcomes in patients with T2DM and may have direct and indirect effects on arterial function.\textsuperscript{46,47} However, in our study no participants were on SGLT-2 inhibitor treatment and only 15% were on GLP-1 receptor agonists. RAS inhibitors, which can improve arterial stiffness, were being taken by 90% of our cohort and use was similar in both treatment groups.

Following 48 weeks of treatment with calcitriol, we observed significant reduction in iPTH consistent with what is observed with active vitamin D treatment in other studies.\textsuperscript{14,41} In contrast no significant changes in 25(OH)D and 1,25(OH)\textsubscript{2}D levels were observed.
during treatment with calcitriol, which has also been observed in other studies.\textsuperscript{14,36} A possible explanation for this result in our study and others may be that calcitriol is acting as a strong inducer of CYP24A1, an enzyme that metabolises/inactivates both 25(OH)D and 1,25(OH)\textsubscript{2}D.\textsuperscript{36}

As described previously, we did not observe any impact of calcitriol on several exploratory secondary endpoints, such as central arterial pressure, augmentation index and albumin excretion that are biomarkers and clinical predictors of enhanced cardio-renal risk. The study was, however, not designed or powered to detect changes in the multiple secondary endpoints. As far as we are aware, data on the impact of vitamin D on central arterial pressures and augmentation index have not been reported in patients with T2DM and CKD. Our findings contrast with results from other studies, which demonstrated some modest effects on arterial stiffness measures; however, this may be due to several factors including participant selection, duration of treatment and choice of vitamin D treatment, as all of these studies were in predominantly nondiabetic CKD people, had much shorter duration of treatment and used different modes of vitamin D replacement.\textsuperscript{13,35–37} With regard to the impact of vitamin D treatment (with active or inactive vitamin D metabolites) on renal endpoints, such as albuminuria or change in eGFR, there are conflicting data. Some studies demonstrate favourable changes whilst others do not, as shown in a recent large long-term study in people with prediabetes, where vitamin D supplementation did not affect progression of kidney disease or have a clinically meaningful effect on changes in albuminuria or eGFR.\textsuperscript{38,49}

There have been concerns about the safety of active vitamin D therapy in predialysis CKD with the risk of hypercalcaemia and potential increased risks of vascular calcification being highlighted.\textsuperscript{36} In one study in patients with CKD stage 3b and 4, higher rates of hypercalcaemia as well as hyperphosphataemia were noted, and 11% of participants in the calcitriol arm commenced on phosphate binders during the study.\textsuperscript{36}

In our study of T2DM with stable stage 3 CKD, however, we did not observe any significant effect of hypercalcaemia or hyperphosphataemia. We speculate that a lack of any significant change in Ao-PWV with 0.25 mcg of daily calcitriol after 48 weeks of treatment suggests that increased vascular calcification (that can manifest as a rise in Ao-PWV) is unlikely at this dose. However, studies with more direct and better measures/indices of vascular calcification are required to confirm this.

Active vitamin D insufficiency is associated with arterial stiffness and adverse cardiovascular and kidney outcomes in people with diabetes and CKD. In animal studies and small short duration trials in people without diabetes who have CKD, vitamin D treatment improves arterial stiffness and function. However our results from a 48 week randomised controlled trial of active vitamin D (calcitriol) in people with T2DM and CKD stage 3 did not demonstrate any significant reduction on Ao-PWV or other indices of central arterial stiffness or albuminuria. These results do not provide evidence to support the use of active vitamin D for improving arterial stiffness and consequent putative cardio-renal protection in people with T2DM and stage 3 CKD.

ACKNOWLEDGEMENTS
The study was funded by a research grant from Diabetes UK (09/0003886). We wish to thank the support and help of Professor Martin Gulliford (School of Population Health & Environmental Sciences, King’s College London) and the trial data monitoring committee, which included Dr Peter Watkins, Dr Phil Chowiencyzk and Dr Richard Hooper. S.A. was funded/supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy’s and St Thomas’ NHS Foundation Trust and King’s College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. We also thank the research nurses and participants who assisted in this work.

COMPETING INTERESTS
The authors declare that there are no conflicts of interest associated with the manuscript.

CONTRIBUTORS
J.K., L.G., N.F. and S.T. designed the study, interpreted the data and drafted the article. N.F., A.P., D.S., M.F. and A.C. contributed and led on data analysis and interpretation. All authors have reviewed the article and approved the final draft.

DATA AVAILABILITY STATEMENT
Requests for access to data should be addressed to the corresponding author.

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SUPPORTING INFORMATION
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How to cite this article: Karalliedde J, Fountoulakis N, Corcillo A, et al. Effect of calcitriol treatment on arterial stiffness in people with type 2 diabetes and stage 3 chronic kidney disease. Br J Clin Pharmacol. 2023;89(1):279-289. doi:10.1111/bcp.15484