Paricalcitol and Endothelial Function in Chronic Kidney Disease Trial
Carmine Zoccali, Giuseppe Curatola, Vincenzo Panuccio, Rocco Tripepi, Patrizia Pizzini, Marica Versace, Davide Bolignano, Sebastiano Cutrupi, Raffaele Politi, Giovanni Tripepi, Lorenzo Ghiadoni, Ravi Thadhani, Francesca Mallamaci

Abstract—Altered vitamin D metabolism and low levels of the active form of this vitamin, 1,25-dihydroxy-vitamin D, is a hallmark of chronic kidney disease (CKD), but there is still no randomized controlled trial testing the effect of active forms of vitamin D on vascular function in patients with CKD. Paricalcitol and Endothelial function in chronic kidney disease (PENNY) is a double-blinded randomized controlled trial (ClinicalTrials.gov, NCT01680198) testing the effect of an active form of vitamin D, paricalcitol (2 μg/d×12 weeks) on endothelium-dependent and endothelium-independent vasodilatation in 88 patients with stage 3 to 4 CKD and parathormone >65 pg/mL (paricalcitol, n=44; placebo, n=44). Paricalcitol treatment reduced parathormone (−75 pg/mL; 95% confidence interval, −90 to −60), whereas parathormone showed a small rise during placebo (21 pg/mL; 95% confidence interval, 5–36). Blood pressure did not change in both study arms. Baseline flow-mediated dilation was identical in patients on paricalcitol (3.6±2.9%) and placebo (3.6±2.9%) groups. After 12 weeks of treatment, flow-mediated dilation rose in the paricalcitol but not in the placebo group, and the between-group difference in flow-mediated dilation changes (the primary end point, 1.8%; 95% confidence interval, 0.3–3.1%) was significant (P=0.016), and the mean proportional change in flow-mediated dilation was 61% higher in paricalcitol-treated patients than in placebo-treated patients. Such an effect was abolished 2 weeks after stopping the treatment. No effect of paricalcitol on endothelium-independent vasodilatation was registered. Paricalcitol improves endothelium-dependent vasodilatation in patients with stage 3 to 4 CKD. Findings in this study support the hypothesis that vitamin D may exert favorable effects on the cardiovascular system in patients with CKD. (Hypertension. 2014;64:00-00.) ●

Online Data Supplement

Key Words: atherosclerosis ■ chronic kidney disease ■ endothelium ■ hypertension ■ paricalcitol ■ vitamin D

Low levels of 1,25-OH vitamin D (1,25-OH2VD) and vitamin D resistance are classical features of chronic kidney disease (CKD), a condition denoting a high risk for cardiovascular complications. Although the use of vitamin D compounds for the prevention and treatment of mineral and bone disorders in CKD is recommended by current guidelines, to date there is no strong evidence showing benefits of these compounds on the cardiovascular system in CKD. In a recent randomized clinical trial that tested the hypothesis that the synthetic-activated form of vitamin D, paricalcitol (paricalcitol, 19-nor-1,25-OH, vitamin D), reduces left ventricular (LV) hypertrophy in patients with CKD, and no regression in LV hypertrophy was registered. Post hoc analyses in this study showed a highly significant reduction in left atrial volume in paricalcitol-treated patients, suggesting that paricalcitol may favorably affect LV diastolic relaxation and distensibility, a function that goes hand in hand with endothelium-dependent vasodilatation. Endothelial cells not only express the vitamin D receptor but also respond to the active form of vitamin D (1,25-OH2VD) with cell-specific gene regulation and functional effects, and activation of the vitamin D receptor may have a favorable influence on the high risk for atherosclerotic complications in CKD. Vascular function, as measured in the brachial artery by the endothelium, nitric oxide (NO)–dependent flow-mediated dilatation (FMD) response to increased shear stress by forearm ischemia, is a potentially relevant surrogate end point in cardiovascular research because it predicts incident risk for cardiovascular events in various settings, including population-based studies and studies in patients with arterial hypertension or myocardial ischemia, and in patients with CKD. With this background in mind, we performed the Paricalcitol and Endothelial function in chronic kidney disease (PENNY) trial, a double-blind, randomized, controlled...
trial, to test whether administration of paricalcitol improves vascular function in patients with stage 3 to 4 CKD.

Methods
The study protocol was approved by the ethics committee of our hospital, and all patients provided written informed consent.

Patients
All patients with stage 3 to 4 CKD who had undergone ≥1 visit in the outpatient clinic of our unit in the calendar year 2010 were considered for this study. Exclusion criteria were age <18 or >80 years, glomerular filtration rate (GFR) <15 or >60 mL/min per 1.73 m², parathormone values <65 pg/mL, serum total Ca <2.2 or >2.5 mmol/L, phosphate levels <2.9 or >4.5 mg/dL, treatment with vitamin D compounds or antiepileptic drugs, neoplasia or symptomatic cardiovascular disease, or liver disease (bilirubin, aminotransferases, and total alkaline phosphatase >3× upper limit of normal). Enrollment started in June 2011 and was completed on May 2012.

Study Design and Treatments
PENNY is a double-blind, randomized, parallel group trial (ClinicalTrials.gov identifier, NCT01680198) in patients with stage 3 to 4 CKD. The patients were enrolled in a nephrology, hypertension, and transplantation unit serving a population of about 1 million residents in the region of Calabria in Southern Italy. During the run-in period, we checked and stabilized concomitant treatments. No placebo was used in this period, and no patient was excluded during the same period. After baseline measurements, patients who met the inclusion criteria were randomized to receive 2 μg paricalcitol capsules (or matching placebo) daily for 12 weeks. This dose was adjusted on the basis of serum parathormone and Ca, and the maximum dose allowed was 2 μg daily. If a subject experienced oversuppression of serum parathormone (defined as a serum parathormone, <15 pg/mL) or hypercalcemia (defined as Ca >2.75 mmol/L), he or she continued to take the study drug at reduced dosage of 1 μg daily every other day and returned in 2 weeks for an unscheduled visit. If in this visit, the parathormone and Ca did not return >15 pg/mL and <2.75 mmol/L, respectively, the drug was discontinued.

Randomization and Masking
Patients were randomized (1:1) to receive 2 μg paricalcitol once daily or matching placebo. Paricalcitol and placebo pills were identical in appearance and in packaging. The investigators and the personal involved in this trial were all blind to treatment allocation. To ensure concealment, packaging was done by the study sponsor and distributed by the study investigators as masked study drug kits. Treatment assignment remained undisclosed till all database issues had been solved.

Procedures
After a 2-week run-in, patients received their assigned treatment for 12 weeks together with medications used for their usual care. Antihypertensive drugs were adjusted in accordance with European Society of Hypertension guidelines. In particular, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers were maintained unmodified throughout the trial. Study visits were scheduled at baseline, at 2-week intervals during the treatment phase, and 2 weeks after treatment withdrawal. Blood pressure (BP) and heart rate measurements, adverse events registration, adherence to treatment and routine biochemistry including parathormone, Ca and P serum, and blood count were performed at every visit. Blood samples for 1,25-OH2VD, 25-OH VD, aldosterone, fibroblast growth factor 23 (FGF23), and aldosterone were taken only at baseline, at 12 weeks, and 2 weeks after treatment withdrawal.

Vascular Function Studies
Vascular function studies were performed in a comfortable, sound insulated, test room with constant temperature (22–24°C). These studies were strictly standardized according to a protocol developed at the coordinating center of a national (Italian) working group of vascular function testing.

Endothelium-dependent and endothelial-independent vasodilatation was assessed in the right brachial artery by a Toshiba Nemio XG machine applying a 7.5 to 10 MHz transducer. After baseline recording (1 minute), a standard BP sphygmomanometer was placed on the right forearm 2 cm below the elbow and the cuff was inflated to 250 mmHg, and this pressure was maintained for 5 minutes. Recordings were then performed during the 4 minutes after cuff deflation to estimate endothelium-dependent, flow-mediated vasodilatation. In studies of endothelium-independent vasodilatation, recording times were 1 minute for the baseline assessment and 5 minutes for changes in arterial diameter brought about by a low dose (25 μg) of sublingual glyceryl trinitrate. FMD and endothelium-independent vasodilatation were computed as the maximal proportional (%) increase in diameter over baseline (mean of measures obtained during the first minute) by an automatic edge detection system. In a multicenter reproducibility study, endothelium-dependent and endothelium-independent vasodilatation by this technique were highly reproducible measurements because in a Bland–Altman analysis, only in 2% of cases, the intra- and intersession differences were outside the agreement limits.
Biochemical Measurements and GFR

Serum calcium, phosphate, glucose, lipids, and high-sensitivity C-reactive protein were measured in the routine clinical pathology laboratory at our institution. Plasma parathormone was measured by immunoradiometric assay (DiaSorin, Stillwater, MN), 25-OH vitamin D and 1,25-OH, vitamin D by radioimmunoassay (Immunodiagnostic Systems, Boldon, United Kingdom), and FGF23 by ELISA (Kainos Laboratories, Bunkyo, Tokyo, Japan). Plasma parathormone was measured by immunoradiometric assay (Immunotech SRO, Prague, Czech Republic) and aldosterone by radioimmunoassay (Immunotech SRO, Marseille Cedex, France). Serum creatinine was measured by the Roche enzymatic, isotope dilution mass spectrometry calibrated, method and serum cystatin C by the Siemens Dade Behring kit, which is traceable to the International Federation of Clinical Chemistry Working Group for Standardization of Serum Cystatin C, and the GFR was calculated by the CKD-Epi creatinine-Cystatin formula.13

Study Power and Statistical Analysis

The primary efficacy measure of PENNY was the change in FMD from baseline to the last measurement during treatment. The trial had 80% power to detect as statistically significant (2-tailed, P<0.05) 2% difference (SD, ±3.0%) in the change in FMD between paricalcitol-treated and paricalcitol-untreated patients (primary outcome measure).

Data analysis for the primary outcome was done by comparing the changes in FMD in paricalcitol-treated and paricalcitol-untreated patients by using the t Test for independent observations. Possible differences in risk factors at baseline not controlled by randomization (ie, differences due to chance) were accounted for by using multivariate analysis. Data analysis was performed by SPSS for Windows (version 9.0; Chicago, IL). We also assessed the change in the primary outcome measure 2 weeks after treatment completion.

Results

Figure 1 shows the consort flow diagram of the study. The source population included all patients with CKD (n=455) who underwent ≥1 visit in the outpatient clinic of our renal unit in the calendar year 2010. Overall, 89 patients (19.6%) were randomized into the study: 45 to paricalcitol and 44 to placebo. One patient (in the active arm) developed dyspnea after 2 weeks and withdrew his consent to continue the study. Because of early withdrawal, this patient performed just the baseline hemodynamic study, and therefore, he could not be included in the final data analysis. As shown in Table 1, patients randomized to paricalcitol and placebo did not differ for demographic, clinical, and biochemical characteristics, but the GFR tended to be higher (P=0.06) in those randomized to paricalcitol. The diagnosis of underlying renal diseases (established by biopsy in patients with glomerulonephritis, on clinical criteria for nephrosclerosis and diabetes mellitus–related CKD, and by renal imaging for adult polycystic kidney disease) did not differ in the paricalcitol and placebo groups (nephrosclerosis, 17 versus 14 patients; diabetes mellitus–related CKD, 11 versus 16 patients; chronic glomerulonephritis, 9 versus 4 patients; polycystic kidney disease, 3 versus 3 patients; and other diagnoses, 4 versus 7 patients). No patient had vitamin D deficiency (25-OH, vitamin D levels <10 ng/mL), but 26 patients in the group randomized to paricalcitol and 19 in the group randomized to placebo (P=0.20) had vitamin D insufficiency (25-OH, vitamin D levels 10 to 19 ng/mL and ≤30 ng/mL, respectively). C-reactive protein was comparable in the 2 groups, and 25% and 36% of patients in the active arm and in the placebo arms had C-reactive protein values ≥3 mg/L, respectively. Overnight proteinuria was absent in 47 patients (22 of the placebo arm and 25 of the active arm). In the remaining cases, overnight proteinuria ranged from 0.02 to 1.82 mg/L in the placebo arm (median, 0.50 mg/L) and from 0.04 to 1.62 mg/L in the active arm (median, 0.26 mg/L), and the difference was largely not significant (P=0.77). Drug treatments were similar between the 2 groups except for calcium carbonate, these latter being more frequently administered in patients on placebo (Table S1 in the online-only Data Supplement).

Effects of Paricalcitol on Biomarkers of Mineral Metabolism, the GFR, and Other Variables

Paricalcitol treatment markedly reduced parathormone (Table 2), which remained substantially unmodified during placebo. Serum calcium and phosphate increased in the paricalcitol group and remained unchanged in the placebo group. FGF23 rose markedly in paricalcitol-treated patients but not in those on placebo. Conversely, serum 1,25-OH, vitamin D levels were suppressed in paricalcitol-treated patients and showed a slight fall in the placebo group. Changes in serum 25-OH, vitamin D did not differ (Table 2).

Table 1. Demographic, Clinical, and Biochemical Characteristics of Patients of the 2 Study Arms

<table>
<thead>
<tr>
<th>Variables</th>
<th>Active Group (n=44)</th>
<th>Placebo Group (n=44)</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>63±11</td>
<td>62±12</td>
<td>0.65</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>59%</td>
<td>70%</td>
<td>0.27</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>12%</td>
<td>19%</td>
<td>0.37</td>
</tr>
<tr>
<td>Past smokers, %</td>
<td>45%</td>
<td>41%</td>
<td>0.66</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29±5</td>
<td>29±5</td>
<td>0.66</td>
</tr>
<tr>
<td>Systolic/diastolic blood pressure, mm Hg</td>
<td>123±16/73±9</td>
<td>129±21/73±11</td>
<td>0.16/0.81</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>67±8</td>
<td>68±10</td>
<td>0.64</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.94±2.55</td>
<td>6.05±1.78</td>
<td>0.84</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.25±1.06</td>
<td>4.20±1.11</td>
<td>0.84</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.22±0.28</td>
<td>1.30±0.34</td>
<td>0.18</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.28±0.88</td>
<td>2.28±0.93</td>
<td>0.91</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.58±0.67</td>
<td>1.34±0.50</td>
<td>0.06</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>168±44.2</td>
<td>212±7.07</td>
<td>0.001</td>
</tr>
<tr>
<td>Cystatin, mg/L</td>
<td>1.98±0.54</td>
<td>2.26±0.69</td>
<td>0.04</td>
</tr>
<tr>
<td>GFRlow, mL/min per 1.73 m²</td>
<td>34±12</td>
<td>29±13</td>
<td>0.06</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>120±20</td>
<td>120±20</td>
<td>0.49</td>
</tr>
<tr>
<td>Calcium, mmol/L</td>
<td>2.25±0.12</td>
<td>2.21±0.10</td>
<td>0.16</td>
</tr>
<tr>
<td>Phosphate, mmol/L</td>
<td>1.20±0.19</td>
<td>1.23±0.16</td>
<td>0.29</td>
</tr>
<tr>
<td>Parathormone, ng/L</td>
<td>102 (81–146)</td>
<td>102 (85–154)</td>
<td>0.70</td>
</tr>
<tr>
<td>FGF23, pg/mL</td>
<td>64.7 (52.7–81.2)</td>
<td>78.0 (57.3–107)</td>
<td>0.07</td>
</tr>
<tr>
<td>1,25-OH vitamin D, pmol/L</td>
<td>104±41.6</td>
<td>93±41.6</td>
<td>0.32</td>
</tr>
<tr>
<td>25-OH vitamin D, mmol/L</td>
<td>82±39.9</td>
<td>94±39.9</td>
<td>0.19</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>1.18 (0.68–3.02)</td>
<td>2.49 (0.99–3.74)</td>
<td>0.11</td>
</tr>
<tr>
<td>PRA, μg/L per hour</td>
<td>47.6 (15.3–118.5)</td>
<td>27.6 (13.4–78.9)</td>
<td>0.35</td>
</tr>
<tr>
<td>Aldosterone, nmol/L</td>
<td>4.93±3.10</td>
<td>5.65±3.13</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD, median and interquartile range, or as percentage frequency, as appropriate. BMI indicates body mass index; FGF23, fibroblast growth factor 23; GFR, glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and PRA, plasma renin activity.
GFR reduced by 3.8 mL/min per 1.73 m² in paricalcitol-treated patients (95% confidence interval [CI], −5.2 to −2.5 mL/min per 1.73 m²), a figure that significantly differed (between-group difference in baseline to 12-week change, P<0.001) from that observed in the control arm (−0.6 mL/min per 1.73 m²; placebo, −1.2 mL/min per 1.73 m²; 95% CI, −1.8 to 0.5 mL/min per 1.73 m²; Table 3). No difference between the active and the placebo arms was found at 12 weeks (P=0.77) for changes in proteinuria (paricalcitol-treated patients, 0.23 mg/L; 95% CI, 0.07–0.39 mg/L versus placebo group, 0.30 mg/L; 95% CI, −0.14 to −0.74 mg/L). Two weeks after stopping the study drugs (ie, at 14 weeks), GFR rose in patients who had been on paricalcitol and remained the same in those who had been on placebo so that the corresponding baseline–14th week changes (paricalcitol, −1.6 mL/min per 1.73 m²; 95% CI, −2.9 to −0.3 mL/min per 1.73 m²; placebo, −1.2 mL/min per 1.73 m²; 95% CI, −2.5 to 0.05 mL/min per 1.73 m²) were almost identical (P=0.71; Table 3).

No significant differences were registered for low- and high-density lipoprotein cholesterol, triglycerides, and glucose (data not shown, P=0.33–0.91). Changes in plasma levels of renin, aldosterone, and C-reactive protein did not differ in paricalcitol and placebo-treated patients (Table 2).

Vascular Function
At 12 weeks, changes in BP (paricalcitol, −1.0 mm Hg; 95% CI, −3.0 to 1.0 mm Hg; placebo, −3.0 mm Hg; 95% CI, −6.0 to 0.2 mm Hg, P=0.40) did not differ between the two groups. Heart rate increased on average 1 bpm (95% CI, −1 to 3 bpm) in paricalcitol-treated patients and reduced 3 bpm in patients on placebo (95% CI, −6 to 1 bpm).

At baseline (week 0), the FMD response to ischemia was identical in the two groups. FMD increased to 4.5±3.4% in paricalcitol-treated patients but not in those on placebo (Figure 2), and at 12th week, the FMD was significantly higher in paricalcitol-treated patients (P=0.013). Accordingly, after 12 weeks of treatment, the between-group difference in FMD changes (the primary end point, 1.8%; 95% CI, 0.3–3.1%) was statistically significant (P=0.016), and the mean proportional change in FMD was 61% higher in paricalcitol-treated patients than in placebo-treated patients (Figure 2). The difference in FMD changes remained unmodified (P=0.012) after adjustment for baseline GFR and use of phosphate binders (ie, the variables which significantly differed between the 2 study arms at baseline). Furthermore, the proportion of patients where FMD increased was substantially higher in the paricalcitol group (28/44) than in the placebo group (15/44; χ²=7.7, P=0.006).

Table 3. GFR Throughout the Study Periods in the 2 Study Arms

<table>
<thead>
<tr>
<th>Variable</th>
<th>Arms</th>
<th>0 wk</th>
<th>12th wk</th>
<th>Difference (12th vs 0 wk)</th>
<th>14th wk</th>
<th>Difference (14th vs 0 wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR, mL/min per 1.73 m²</td>
<td>Placebo</td>
<td>29±13</td>
<td>28±14</td>
<td>−0.6 (−1.8 to 0.5)</td>
<td>28±13</td>
<td>−1.2 (−2.5 to 0.05)</td>
</tr>
<tr>
<td></td>
<td>Active arm</td>
<td>34±12</td>
<td>30±12</td>
<td>−3.8 (−5.2 to −2.5)</td>
<td>33±13</td>
<td>−1.6 (−2.9 to −0.3)</td>
</tr>
<tr>
<td></td>
<td>Between-group difference: −3.2 (−4.9 to −1.4), P&lt;0.001</td>
<td>Between-group difference: −0.4 (−2.2 to 1.5)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

At 12th wk, there was a significantly more pronounced decline in the glomerular filtration rate (GFR) in the active arm of the study. The between-group difference in the GFR disappeared 2 wk after stopping paricalcitol at 14th wk. Absolute data within groups are given as mean±SD. Within- and between-group differences are expressed as mean and 95% confidence intervals.
The brachial artery diameter and shear stress remained substantially constant throughout the trial (Table S2). Endothelium-independent vasodilation (the response to glyceryl trinitrate) was unaffected by paricalcitol (paricalcitol, +0.6±4.5%; placebo, +0.3±3.1%; \( P = 0.71 \)). Two weeks after treatment discontinuation, there was no between-group difference (\( P = 0.33 \)) in FMD and the corresponding baseline–14th-week change in FMD in paricalcitol-treated patients (+0.40%; 95% CI, −0.73% to 1.54%) and in placebo-treated patients (−0.15%; 95% CI, −1.12% to 0.82%) did not differ (\( P = 0.46 \); Figure 2).

In a secondary analysis testing for effect modification by variables that may plausibly affect the response to paricalcitol, there was no effect modification by sex, age, diabetes mellitus, BP, and the diagnosis of renal disease. High serum phosphate attenuated the FMD rise associated with paricalcitol treatment (\( P = 0.002 \)). No similar interaction emerged with other biomarkers of mineral metabolism (parathormone, FGF23, 1,25-OH\(_2\) V\(_D\), and 25-OH V\(_D\)), and Ca).

**Adverse Effects of Paricalcitol**

One patient developed an episode of dyspnea 2 after starting paricalcitol and dropped out of the study. Two patient in the paricalcitol group developed frank hypercalcemia (serum Ca, >2.75 mmol/L) at 6th and 12th weeks (ie, at the study end). In the first serum, calcium normalized after dose reduction to 1 \( \mu g/d \). Dose reduction was also applied to 1 patient at the 10th week because his serum parathormone was <15 pg/mL. No patient died. Five patients were hospitalized during the study, 4 patients in the paricalcitol arm (1 for elective coronary angiography, 1 for diagnostic studies to clarify anemia worsening, and 2 because chronic obstructive pulmonary disease worsening) and 1 in the placebo arm (anemia worsening). Two patients (1 per study arm) developed mild pedal edema. Two in the placebo arm had pruritus and paresthesias, respectively, whereas 2 in the paricalcitol arm had stypsis and gastric pain, respectively.

**Discussion**

In this randomized clinical trial in patients with stage 3 to 4 CKD, paricalcitol improved endothelium-dependent vasodilatation without modifying endothelium-independent vasodilatation. Such an effect was accompanied by a slight fall in the GFR, and both the improvement in endothelium-dependent vasodilatation and the reduction in the GFR returned toward baseline after drug withdrawal implying that the functional effect of paricalcitol on the endothelium and the GFR was real and reversible. Paricalcitol was well tolerated.

Vascular function studies in man are biologically relevant because disturbed endothelium-dependent vasoregulation precedes structural alterations in the atherosclerosis process,\(^{16}\) while endothelial function improvement preludes to anatomic improvement in animal models.\(^{17}\) Studying the response of local (forearm) blood flow to stimuli that cause NO release such as increased shear stress or acetylcholine is now an established method in cardiovascular research.\(^{10}\) In the present study, we studied vascular function according to a rigorous protocol, including operator training, standardized experimental procedures, and automated B-mode image edge detection system, which provided accuracy and time-dependent reproducibility in a multicenter, nationwide, setting.\(^{14}\)

Endothelial function is markedly deranged in patients with CKD,\(^{11,18}\) and reduced flow-mediated vasodilatation predicts a high risk for cardiovascular events in patients with CKD both predialysis\(^{11}\) and after the start of regular dialysis treatment.\(^{12}\) Alterations in mineral metabolism may play a major role in deranged endothelial function in patients with end-stage CKD because changes in 25-OH V\(_D\), serum phosphate, and FGF23 levels brought about by restored renal function after kidney transplantation correlate with improved endothelium-dependent vasodilatation.\(^{19}\) Cross-sectional studies associated 25-OH V\(_D\), 1,25-OH\(_2\) V\(_D\), and endothelium-dependent vasodilation in patients with stage 3 to 4 CKD\(^{20}\) and in patients with end-stage kidney disease on dialysis,\(^{21}\) suggesting that vitamin D insufficiency or deficiency may have adverse effects on the vascular system.

Paricalcitol was associated with improved survival in large-scale observational studies in patients with end-stage kidney disease.\(^{22}\) Paricalcitol is a synthetic analog of 1,25-OH\(_2\) V\(_D\) endowed with several actions impinging on vascular function.

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**Figure 2.** Endothelium-dependent (flow-mediated dilation [FMD]) vasodilation at baseline, after 12 weeks of treatment, and 2 weeks after stopping treatment (14th weeks) in patients receiving paricalcitol and placebo. Data are means±SD. At 12th and 14th weeks, we also reported (top) the mean proportional change in FMD induced by paricalcitol (PCT) vs placebo group expressed as percentage.
Vitamin D receptor activation by this compound ameliorates endothelium-dependent vasodilatation in subtotally nephrectomized rats, an effect which is completely independent of BP and parathormone levels. As in previous studies applying the same technique, patients with CKD enrolled in this study exhibited endothelial dysfunction. Paricalcitol elicited a coherent rise in the endothelium-dependent FMD response to ischemia in these patients with a 61% increase versus placebo. Importantly, like in the vast majority of nonpharmacological and pharmacological trials with other agents, the effect of paricalcitol was specific, that is, it was confined to FMD leaving unaffected endothelium-independent vasodilatation (the response to nitroglycerine). Furthermore, the effect of paricalcitol was of the same order of that prompted by drugs of proven efficacy in atherosclerosis prevention like drugs interfering with the rennin–angiotensin system, calcium antagonists, and statins. In keeping with a randomized trial testing the effect of paricalcitol on albuminuria in patients with diabetes mellitus, we found that this drug produces a slight, fully reversible reduction in the GFR which in our study was independent of BP. This functional effect may depend on an interference of paricalcitol with the regulation of glomerular microcirculation by the NO system. Of note, the increase in endothelium-dependent vasodilatation in our study occurred in the face of a doubling in plasma levels of FGF23, a biomarker that was associated to endothelial dysfunction in this population.

Observational studies in patients with CKD, like in the Chronic Renal Insufficiency Cohort (CRIC) study, associated high FGF23 with adverse cardiovascular and renal outcomes. However, a recent meta-analysis showed that in patients with CKD, paricalcitol is a safe drug with favorable effect on proteinuria. Like in Vitamin D Receptor Activation With Paricalcitol for Reduction of Albuminuria (VITAL) and in Paricalcitol Capsule Benefits in Renal Failure–Induced Cardiac Morbidity (PRIMO), we found that paricalcitol was well tolerated. Furthermore, in VITAL and PENNY trials, paricalcitol induced favorable changes in biomarkers of the risk for atherosclerotic complications like albuminuria in VITAL and FMD in our study, while a post hoc analysis in PRIMO showed a marked reduction left atrial volume (ie, a sensitive indicator of diastolic dysfunction).

Along with its fundamental role in vascular control, NO is also a key player in LV diastolic function regulation because NO released by the endothelium in coronary arteries accelerates LV relaxation and increases LV distensibility. A strong association between diastolic function and endothelium-dependent vasodilatation has been repeatedly confirmed. Left atrial volume, the sole echocardiographic parameter to be favorably affected by paricalcitol in the PRIMO study, largely reflects LV diastolic function. Vitamin D receptor activation increases LV lusitropy and produces parallel beneficial effects on endothelium-dependent vasodilatation and LV disorders in subtotally nephrectomized rats. Thus, the beneficial effect of paricalcitol on the primary outcome measure (endothelium-dependent vasodilatation) in the present clinical trial fits well with experimental observations in the rat as well as with post hoc analyses in PRIMO trial.

Some limitations related with the generalizability of our findings should be clearly acknowledged. Ours is a single-center study in an ethnically homogeneous population and including a relatively small proportion of participants with diabetes mellitus. Vitamin D effects may vary by race and diabetes mellitus. Furthermore, like in most studies similar to ours, we excluded use of concurrent vitamin D treatment while in clinical practice, and nonactivated forms of vitamin D are prescribed to patients with CKD and vitamin D deficiency or insufficiency. In conclusion, paricalcitol improves endothelium-dependent vasodilatation in patients with stage 3 to 4 CKD. Such an effect indicates that endothelial dysfunction is at least in part a reversible phenomenon in this population.

**Perspectives**

Because improvement in endothelial dysfunction preludes to atherosclerosis regression in primates and signals a reduction in the risk of cardiovascular events, findings in this study represent proof of concept that paricalcitol favorably affects a biological phenomenon of major relevance for atherosclerosis in humans and add new experimental evidence in support of the hypothesis that vitamin D may exert favorable effects on the cardiovascular system in patients with CKD.

**Acknowledgments**

The lead investigator (C. Zoccali) designed the study and oversaw conduct and management of the trial and with the study statistician (G. Tripepi) performed data analysis and interpretation. R. Tripepi performed all vascular function studies with the help of M. Versace, G. Curatola was the clinical coordinator of the study and D. Bolignano, F. Mallamaci, and V. Panuccio recruited and followed up patients participating in this study. P. Pizzini and S. Cutrupi performed hormone measurements. R. Politi was responsible for the electronic database of the study. R. Thadhani was consulted by the lead investigator for study design. The lead investigator wrote the first draft of the article and was responsible for incorporating into the article comments and suggestions by other investigators. All investigators made a critical revision of the article, gave important intellectual contribution, and approved the final version of the article. L. Ghidoni is the coordinator of the national project of endothelial function standardization studies. He was responsible for overseeing all vascular function studies made by R. Tripepi and for critically reading and improving the article.

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This study was supported by a grant of Abbott SRL. The study was designed by the first author (C. Zoccali) and approved by the funding company (Abbot). The sponsor did not participate in data collection and in the statistical analysis and writing of the article. The first author made the final decision to submit for publication. The sponsor had an opportunity to review the article, but all decisions about the final manuscript were made by the study investigators. All authors vouch for the integrity of the data.

**Disclosures**

C. Zoccali received honoraria for lectures from Abbott, Amgen, Genzyme, Roche, and Shire, and his institution received funding from Abbott for the PENNY study. F. Mallamaci received honoraria for lectures from Amgen and Shire and G. Tripepi received support from Abbott. R. Thadhani received a research grant from Abbott Laboratories to the Massachusetts General Hospital and speaker's fees and travel support from Abbott Laboratories. The other authors report no conflicts.

**References**

3. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. KDIGO clinical practice guideline for the diagnosis,


Novelty and Significance

What Is New?

- The Paricalcitol and ENDothelial function in chronic kidney Disease trial (PENNY) trial is the first randomized clinical trial testing the effect of an active form of vitamin D on endothelial function as measured by flow-mediated vasodilatation in patients with stage 3 to 4 chronic kidney disease.

What Is Relevant?

- Paricalcitol improves endothelium-dependent vasodilatation in patients with stage 3 to 4 chronic kidney disease. As endothelial dysfunction predicts incident risk for cardiovascular events, paricalcitol may have a beneficial effect on atherosclerosis in these patients.

Summary

Paricalcitol improves endothelium-dependent vasodilatation but leaves unaffected endothelium-independent (nitroglycerin induced) vasodilatation in patients with stage 3 to 4 chronic kidney disease. Findings in this study support the hypothesis that vitamin D may exert favorable effects on the cardiovascular system in patients with chronic kidney disease.
Paricalcitol and Endothelial Function in Chronic Kidney Disease Trial
Carmine Zoccali, Giuseppe Curatola, Vincenzo Panuccio, Rocco Tripepi, Patrizia Pizzini, Marica Versace, Davide Bolignano, Sebastiano Cutrupi, Raffaele Politi, Giovanni Tripepi, Lorenzo Ghiadoni, Ravi Thadhani and Francesca Mallamaci

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THE PARICALCITOL AND ENDOTHELIAL FUNCTION IN CHRONIC KIDNEY DISEASE TRIAL (PENNY)

Zoccali Carmine¹ ², Curatola Giuseppe², Panuccio Vincenzo¹ ², Tripepi Rocco², Pizzini Patrizia², Versace Marica², Bolignano Davide², Cutrupi Sebastianò², Politi Raffaele², Tripepi Giovanni², Ghiadoni Lorenzo³, Thadhani Ravi⁴, Mallamaci Francesca¹ ².

¹ Nephrology, Hypertension and Renal Transplantation Unit, Ospedali Riuniti, Reggio Cal. Italy. ²CNR-IBIM/IFC Clinical Epidemiology and Pathophysiology of Renal Diseases and Hypertension ³Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy; ⁴Department of Medicine, Division of Nephrology, Massachusetts General Hospital, Boston, Massachusetts, USA

SHORT TITLE: PARICALCITOL IN CKD

WORD COUNT: 6000

Correspondence
Prof. Carmine Zoccali, FASN FNKF, FERA, CNR-IBIM/IFC Clinical Epidemiology and Pathophysiology of Renal Diseases and Hypertension, Ospedali Riuniti
c/o EUROLINE DI BARILLA' FRANCESCA
VIA Vallone Petrara 55-57,
89124 Reggio Calabria, ITALY

E-mail: carmine.zoccali@tin.it
Telephone: 0039 0965 3970210
(cellular phone 0039 3407354062)
FAX: 0039 0965 26879
Table S1: Drug treatments in the two study arms.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Active group (n=44)</th>
<th>Placebo group (n=44)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE inhibitors (%)</td>
<td>47.7%</td>
<td>47.7%</td>
<td>1.00</td>
</tr>
<tr>
<td>Angiotensin Receptor Blockers (%)</td>
<td>59.1%</td>
<td>47.7%</td>
<td>0.29</td>
</tr>
<tr>
<td>Diuretics (%)</td>
<td>34.1%</td>
<td>47.7%</td>
<td>0.19</td>
</tr>
<tr>
<td>Alpha and Beta Blockers (%)</td>
<td>36.4%</td>
<td>38.6%</td>
<td>0.83</td>
</tr>
<tr>
<td>Calcium Antagonists (%)</td>
<td>40.9%</td>
<td>52.3%</td>
<td>0.29</td>
</tr>
<tr>
<td>Statins (%)</td>
<td>45.5%</td>
<td>52.5%</td>
<td>0.52</td>
</tr>
<tr>
<td>Omega 3 polyunsaturated fatty acids (%)</td>
<td>27.3%</td>
<td>11.4%</td>
<td>0.06</td>
</tr>
<tr>
<td>Hypoglycemizing agents (%)</td>
<td>6.8%</td>
<td>18.2%</td>
<td>0.11</td>
</tr>
<tr>
<td>Insulin (%)</td>
<td>13.6%</td>
<td>15.9%</td>
<td>0.76</td>
</tr>
<tr>
<td>Antiplatelet agents (%)</td>
<td>50.0%</td>
<td>54.5%</td>
<td>0.83</td>
</tr>
<tr>
<td>Nitrates (%)</td>
<td>9.1%</td>
<td>4.5%</td>
<td>0.40</td>
</tr>
<tr>
<td>Erythropoietin stimulating agents (%)</td>
<td>2.3%</td>
<td>2.0%</td>
<td>0.56</td>
</tr>
<tr>
<td>Iron (%)</td>
<td>11.4%</td>
<td>13.6%</td>
<td>0.75</td>
</tr>
<tr>
<td>Calcium carbonate (%)</td>
<td>0.0%</td>
<td>22.7%</td>
<td>0.003</td>
</tr>
<tr>
<td>Proton pump inhibitors (%)</td>
<td>63.6%</td>
<td>50.0%</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Data are expressed as percent frequency and compared by the Chi Square Test (with the continuity correction when appropriate).
Table S2 Pre-ischemia brachial diameter and shear rate in the active and the control arm. None of the differences was significant implying maintained comparability of the pre-ischemia parameters throughout the trial.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Arms</th>
<th>0 week</th>
<th>12th week</th>
<th>Difference (12th week versus 0 week)</th>
<th>14th week</th>
<th>Difference (14th week versus 0 week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brachial diameter</td>
<td>Placebo</td>
<td>4.9±1.0</td>
<td>5.1±0.9</td>
<td>0.12(-0.06 to 0.31)</td>
<td>5.0±0.8</td>
<td>0.17(-0.05 to 0.39)</td>
</tr>
<tr>
<td></td>
<td>Active arm</td>
<td>4.7±1.0</td>
<td>4.8±0.9</td>
<td>0.09(-0.08 to 0.27)</td>
<td>4.9±1.0</td>
<td>0.23(0.05 to 0.40)</td>
</tr>
<tr>
<td></td>
<td>Between groups difference</td>
<td>-0.03 (-0.22 to 0.28)</td>
<td>Between groups difference</td>
<td>0.06 (-0.22 to 0.34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shear rate (sec⁻¹)</td>
<td>Placebo</td>
<td>210±119</td>
<td>209±77</td>
<td>-0.8(-36.1 to 34.6)</td>
<td>219±83</td>
<td>9.8(-28.6 to 48.2)</td>
</tr>
<tr>
<td></td>
<td>Active arm</td>
<td>212±69</td>
<td>240±105</td>
<td>28.3 (-1.4 to 58.0)</td>
<td>256±123</td>
<td>43.9 (7.0 to 80.8)</td>
</tr>
<tr>
<td></td>
<td>Between groups difference</td>
<td>29.1 (-16 to 75)</td>
<td>Between groups difference</td>
<td>34.0 (-18 to 87)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Absolute data within groups are given as mean ± standard deviation. Within and between groups differences are expressed as mean and 95% confidence intervals.