Moderate salt restriction with or without paricalcitol in type 2 diabetes and losartan-resistant macroalbuminuria (PROCEED): a randomised, double-blind, placebo-controlled, crossover trial

Aneliya Parvanova, Matias Trillini*, Manuel A Podestà*, Ilian Petrov Iliev, Barbara Ruggiero, Manuela Abbate, Annalisa Perna, Francesco Peraro, Olimpia Diadei, Nadia Rubis, Flavio Gaspari, Fabiola Carrara, Nadia Stucchi, Antonio Belviso, Antonio C Bossi, Roberto Trevisan, Giuseppe Remuzzi, Martin de Borst†, Piero Ruggenenti†, on behalf of the PROCEED Study Organization and the Scientific Writing Academy (SWA) 2016‡*

Summary

Background Macroalbuminuria predicts renal and cardiovascular events in patients with type 2 diabetes. We aimed to assess the albuminuria-lowering effects of salt restriction, paricalcitol therapy, or both, in this population.

Methods In this randomised, double-blind, placebo-controlled, crossover trial, we recruited adult patients with type 2 diabetes from six diabetology outpatient clinics in northern Italy, with 24 h albuminuria of more than 300 mg despite 100 mg per day losartan therapy, blood pressure of less than 140/90 mm Hg, serum creatinine concentration of less than 2 mg/dL, stable renal function on stable renin-angiotensin system inhibitor therapy with a fixed dose of losartan, parathyroid hormone concentration of 20 pg/mL to <110 pg/mL, serum calcium concentration of less than 9·5 mg/dL, and serum phosphate concentration of less than 5 mg/dL, who had been more than 80% compliant with placebo treatment during a 1 month placebo run-in. We allocated patients 1:1 with computer-generated randomisation to an open-label 3 month high-sodium (>200 mEq [4·8 g] per day) or low-sodium (<100 mEq [2·4 g] per day) diet and, within each diet group, to a 1 month double-blind treatment period of oral paricalcitol (2 µg per day) or placebo, followed by 1 month of placebo washout and then a further 1 month double-blind treatment period of paricalcitol or placebo in which patients crossed over to the opposite treatment period. The primary outcome was 24 h albuminuria (median of three consecutive measurements). Analyses were modified intention-to-treat (including all randomly allocated patients who took at least one dose of study drug and had an efficacy measurement after the first treatment period). Patients and investigators were masked to paricalcitol and placebo assignment. Those assessing outcomes were masked to both study drug and diet assignment. This study is registered with ClinicalTrials.gov, number NCT01393808, and the European Union Clinical Trials Register, number 2011-001713-14.

Findings Between Dec 13, 2011, and Feb 17, 2015, we randomly allocated 57 (50%) patients to a low-sodium diet (28 [49%] to paricalcitol then placebo and 29 [51%] to placebo then paricalcitol) and 58 (50%) to a high-sodium diet (29 [50%] to paricalcitol then placebo and 29 [50%] to placebo then paricalcitol). In the low-sodium group (30 mEq of daily sodium intake reduction, equivalent to approximately 1·7–1·8 g per day), 24 h albuminuria was reduced by 36·6% (95% CI 28·5–44·9) from 724 mg (441–1233) at baseline to 481 mg (289–837) at month 3 (p<0·0001), but no significant change occurred in the high-sodium group (from 730 mg [416–1227] to 801 mg [441–1365]; 2·9% [–16·8 to 16·4] increase; p=0·50). Changes between diet groups differed by 32·4% (17·2–48·8; p<0·0001) and correlated with changes in natriuresis (r=0·43; p<0·0001). On the high-sodium diet, paricalcitol reduced the salt-induced albuminuria increase by 17·8% (3·9–32·3) over the month of treatment compared with placebo (p=0·02), whereas on the low-sodium diet, paricalcitol did not have a significant effect versus placebo (increase of 4·1% [–9·3 to 21·6]; p=0·59). During placebo treatment, albuminuria decreased with the low-sodium diet (p=0·0002) and did not significantly change with the high-sodium diet, but changes were significantly different between diet groups (p=0·0004). Treatment was well tolerated and no patients withdrew from the study because of treatment-related effects. 67 adverse events occurred in 52 (45%) patients during paricalcitol treatment and 44 events occurred in 36 (31%) patients during placebo treatment. During paricalcitol therapy, 14 cases of hypercalciuria, six cases of hypercalcaemia, and five cases of hyperphosphataemia were reported in one patient each, all of which were possibly treatment related. One case of hypercalciuria was reported in one patient during the placebo treatment period. One stroke and one coronary event occurred during paricalcitol therapy. No patients died during the study.

Interpretation In patients with macroalbuminuria and type 2 diabetes, moderate salt restriction enhances the antialbuminuric effect of losartan, an effect that could be nephroprotective and cardioprotective in the long term. The finding that paricalcitol prevents a sodium-induced increase in albuminuria provides support for trials to test the long-term risk-benefit profile of paricalcitol add-on therapy in patients with type 2 diabetes and macroalbuminuria refractory to dietary salt restriction, including patients refractory to even moderate salt restriction.

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*Contributed equally (joint first authors)

†Contributed equally (joint last authors)

‡Members listed in the appendix

Istituto di Ricovero e Cura a Carattere Scientifico—Istituto di Ricerche Farmacologiche Mario Negri, Centro Anna Maria Astori, Science and Technology Park Kilometro Rosso, Bergamo, Italy (Prof G Remuzzi FRCP, P Ruggenenti MD, M Trillini MD, A Parvanova DSc, M A Podestà MD, I P Iliev MD, B Ruggiero MD, M Abbate MSc, A Perna MSc, F Peraro StatSciD, O Diadei ChemD, N Rubis ResN, F Gaspari ChemD, F Carrara, N Stucchi); **Department of Medicine, Unit of Nephrology and Dialysis** (G Remuzzi, P Ruggenenti, M A Podestà) **and Unit of Diabetology** (R Trevisan MD), **Azienda Socio-Sanitaria Territoriale Papa Giovanni XXIII, Bergamo, Italy; Department of Biomedical and Clinical Science, L Sacco, University of Milan, Milan, Italy** (G Remuzzi); **Diabetes Clinic, Azienda Socio-Sanitaria Territoriale Bergamo Ovest, Brembate Sopra, Italy** (A Belviso MD); **Endocrine Diseases and Diabetes Unit, Azienda Socio-Sanitaria Territoriale Bergamo Ovest, Treviglio-Caravaggio-Romano, Italy** (A C Bossi MD); **and Department of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands** (M de Borst MD)

Correspondence to:
Prof Giuseppe Remuzzi, Istituto di Ricovero e Cura a Carattere Scientifico—Istituto di Ricerche Farmacologiche Mario Negri, Centro Anna Maria Astori, Science and Technology Park Kilometro Rosso, 24126 Bergamo, Italy
giuseppe.remuzzi@marionegri.it

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Research in context

Evidence before this study

We searched PubMed and the Cochrane Library for articles published up to July 9, 2017, in any language, using the search terms ("diabetic nephropathy/ies" OR ["diabetic" AND "nephropathy/ies"] OR "macroalbuminuria") AND ("sodium, dietary" OR ["sodium" AND "dietary"]) in combination with (["dietary sodium" OR "sodium"] AND ["diet" OR "diet"]); alternatively we used the search terms ("paricalcitol" OR "vitamin D" OR "cholecalciferol" OR "colecalciferol" OR "hydroxycholecalciferol" OR "hydroxycolecalciferol" OR "dihydroxycholecalciferol" OR "dihydroxycolecalciferol" OR "dihydrotachysterol" OR "maxicalcitol" OR "oxacalcitriol" OR "doxercalciferol" OR "dihydroxyvitamin" OR "falecalcitriol" OR "calcitriol" OR "alfacalcidol" OR "alphacalcidol" OR "calcifedol" OR "calcipotriol" OR "epicalcitriol" OR "lexacalcitol" OR "seocalcitol" OR "tacalcitol" OR "ergocalciferol"). We found one prospective study showing that salt intake restriction reduced albuminuria in patients with macroalbuminuria and type 2 diabetes without renin–angiotensin system inhibitor therapy. Two of six additional prospective studies designed to assess the blood pressure (BP)-lowering effect of salt restriction in patients with diabetes found a reduction in albuminuria in patients with type 2 diabetes with microalbuminuria, whereas four other studies, including two in patients with type 1 diabetes, found no treatment effect. One prospective study found a similar albuminuria-lowering effect of dietary salt restriction as compared with hydrochlorothiazide in patients with type 2 diabetes with microalbuminuria or macroalbuminuria and concomitant lisinopril therapy. We found no study exploring the antialbuminuric effect of salt restriction in patients with macroalbuminuria and type 2 diabetes on angiotensin receptor blocker (ARB) therapy. Studies of vitamin D or its analogues included patients with chronic kidney disease-related abnormalities in mineral metabolism. Only two studies primarily assessed the effect of paricalcitol on albuminuria in patients with diabetes. The first found an antialbuminuric effect of treatment in patients with type 1 diabetes and microalbuminuria. The second found a borderline significant (p=0·053) effect of 2 μg per day of paricalcitol on urinary albumin-to-creatinine ratio in patients with type 2 diabetes. Finally, one study assessed the effects of salt restriction and paricalcitol combination therapy in patients with macroalbuminuria and non-diabetic chronic kidney disease. Thus, to our knowledge, no study assessed the effects of salt restriction with or without paricalcitol in patients with type 2 diabetes with residual macroalbuminuria despite renin–angiotensin system inhibitor therapy with an angiotensin-converting enzyme inhibitor or ARB. To our knowledge, no study assessed the effect of moderate salt intake restriction that does not affect arterial BP.

Added value of this study

Our data show that even modest dietary salt intake reduction, averaging approximately 1·7–1·8 g per day (equivalent to 30 mEq of sodium per day), might persistently reduce albuminuria in patients with type 2 diabetes at high risk of renal and cardiovascular events because of persistent macroalbuminuria despite full-dose losartan therapy. In the low-sodium group, albuminuria similarly decreased during both treatment periods. In the high-sodium group, albuminuria did not significantly change, but the two trends between treatment groups were significantly different (p=0·02). Moreover, changes in albuminuria in the two sodium diet groups were similar during paricalcitol whereas albuminuria did not significantly change with the high-sodium diet and significantly decreased in the low-sodium diet group during placebo and these trends were significantly different (p=0·0004). Thus, we concluded that paricalcitol protected from the sodium-dependent increase in albuminuria.

Implications of all the available evidence

The albuminuria-lowering effect of salt intake restriction that we observed in patients with macroalbuminuria and type 2 diabetes on losartan could explain the retrospective observation that low salt intake was associated with an improved nephroprotective and cardioprotective effect of ARB therapy in patients with type 2 diabetes with overt nephropathy. Thus, our data provide a rationale for even moderate dietary salt restriction in patients with type 2 diabetes and residual macroalbuminuria despite full-dose losartan. Moreover, less restrictive dietary targets than those recommended by guidelines, which have less effect on food palatability, might achieve larger clinical benefit because of improved patient compliance. These findings could have major clinical implications since patients with macroalbuminuria and type 2 diabetes account for most patients with chronic kidney disease at increased risk of end-stage renal disease and cardiovascular events. Paricalcitol add-on therapy might protect patients with type 2 diabetes who are refractory to dietary restriction from a salt-induced increase in albuminuria. Paricalcitol, however, might have side-effects and randomised clinical trials are needed to test its long-term risk-benefit profile in this context.

Introduction

Diabetic renal disease affects about 35% of patients with diabetes and is the leading cause of end-stage renal disease worldwide.[1] Albuminuria is an early marker and major determinant of progression of the disease[2] and has become an important treatment target for nephroprotection and cardioprotection in patients with diabetes over the past few decades.[3] Renin–angiotensin system (RAS) blockade by angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) delays loss of renal function by reducing blood pressure (BP) and albuminuria in type 1 and type 2 diabetes.[4] However, despite optimally titrated RAS blockers, many patients have residual albuminuria, which is associated with

progressive renal function loss and adverse cardiovascular outcomes.[5,6]

Losartan is standard therapy for patients with type 2 diabetes with macroalbuminuria,[7] and retrospective analyses of randomised trials suggest that its renal and cardiovascular protective effects are strengthened by salt restriction.[8] However, prospective studies assessing the effects of salt restriction on albuminuria in patients with diabetes are scarce and their results conflicting.[9] A short-term crossover study[10] found that 6 weeks of tight salt restriction to 50 mEq intake per day had albuminuria-lowering effects similar to those of hydrochlorothiazide in a mixed population of patients with type 2 diabetes and microalbuminuria or macroalbuminuria already taking 40 mg per day of the ACE inhibitor lisinopril. However, to our knowledge, no study has assessed the effect of moderation of dietary sodium in patients with type 2 diabetes with residual macroalbuminuria despite full-dose ARB therapy.

Vitamin D and its analogues might also be nephroprotective in patients with diabetes. In experimental diabetes, the vitamin D receptor activator paricalcitol in combination with losartan normalised albuminuria and slowed progression of renal damage more effectively than did losartan monotherapy,[11] through mechanisms involving a protective action on podocytes, reduced renin concentrations, and attenuated inflammation and fibrosis.[12] A crossover study[13] in patients with type 1 diabetes with residual macroalbuminuria despite treatment with ACE inhibitors or ARBs found that paricalcitol reduced albuminuria. The VITAL trial[14] found that 2 µg per day of paricalcitol achieved a urinary albumin-to-creatinine ratio (UACR) reduction versus placebo in patients with type 2 diabetes with a baseline UACR in first morning void of 11–339 mg/mmol despite losartan (p=0·053). Post-hoc analyses found that this effect was almost fully driven by 29 patients with natriuresis exceeding 178 mEq per day (equivalent to a daily salt intake exceeding approximately 4·5 g), whereas no treatment effect was observed in patients with sodium excretion lower than this level.

Thus, we designed a prospective, randomised controlled trial to assess the albuminuria-lowering effect of salt intake restriction in patients with type 2 diabetes with residual macroalbuminuria despite stable full-dose losartan therapy, and to assess whether and to what extent salt intake modulates the effect of paricalcitol on urinary albumin as compared with placebo in this context (appendix).

Methods

Study design and participants

In this randomised, double-blind, placebo-controlled, crossover trial, we recruited patients from six diabetology outpatient clinics in the Bergamo Province, Italy. We identified potentially eligible adult patients with type 2 diabetes, macroalbuminuria, and concomitant RAS inhibitor therapy by assessment of their clinical records at the diabetology units involved in the study. Those who consented to study participation, stopped previous RAS inhibitor therapy and started treatment with a fixed dose (100 mg per day) of losartan were maintained on their usual dietary sodium intake and entered a 1 month run-in placebo treatment period. At the end of the run-in period, we did baseline assessments and included patients with 24 h albuminuria of more than 300 mg (median of three consecutive measurements), BP of less than 140/90 mm Hg, serum creatinine concentration of less than 2 mg/dL, stable renal function and more than 80% compliance to the fixed dose (100 mg per day) of losartan during the run-in period who had a parathyroid hormone (PTH) concentration of 20 pg/mL to less than 110 pg/mL, a serum calcium concentration of less than 9·5 mg/dL, and a serum phosphate concentration of less than 5 mg/dL. We excluded patients if they had received vitamin D or a vitamin D analogue in the previous 3 months, were taking any drug known to affect bone metabolism, had a history of kidney stones, had an HbA_{1c} concentration of greater than 12% (108 mmol/mol), had evidence of vitamin D toxicity, had a contraindication to paricalcitol, had any clinically relevant condition that might affect study participation or results, or were pregnant or lactating or women of childbearing potential not using contraception.

The Clinical Research Center (CRC) for Rare Diseases "Aldo e Cele Daccò" of the Istituto di Ricovero e Cura a Carattere Scientifico Mario Negri Institute for Pharmacological Research, Ranica, Italy, coordinated and monitored the study and processed all laboratory samples. The ethics committees of participating centres approved the study, which was done in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. We obtained written informed consent from all patients before enrolment. The study protocol is available online.

For the **study protocol** see http://clintrials.marionegri.it/images/Trials/protocol_proceed.pdf

Randomisation and masking

After initial assessment of eligibility and the 1 month placebo run-in period, we randomly allocated those who fulfilled the selection criteria and showed at least 80% compliance to placebo treatment during the run-in phase 1:1 to a low-sodium or high-sodium diet. Dietary allocation was by necessity open, but those assessing outcomes were masked to diet group. Within each diet group, we also randomly allocated patients 1:1 to paricalcitol or placebo, followed by a 1 month placebo washout period, and then patients crossed over to the other treatment for a further 1 month of paricalcitol or placebo. A statistician not directly involved in the trial allocated patients by use of computer-generated randomisation. Medication containers were labelled with a unique number representing the randomly allocated study sequence. Placebo capsules had identical appearance, smell, and taste to paricalcitol capsules. Randomisation to paricalcitol or placebo was double-blind (ie, patients, investigators,

and those assessing outcomes were masked to study drugs).

Procedures

Patients in the low-sodium group consumed less than 100 mEq (2·4 g) sodium per day for 3 months compared with more than 200 mEq (4·8 g) per day in the high-sodium group. Patients also received paricalcitol (19-nor-1,25[OH]$_2$-vitamin D$_2$; AbbVie, Ludwigshafen, Germany) 2 µg oral capsules or placebo once daily for 1 month periods. We emphasised the importance of adherence to dietary guidelines[7] to all patients. After diet allocation, however, we gave personally tailored diets to each patient to increase or decrease their salt intake according to target natriuresis (detail of diet prescription and monitoring are provided in the appendix). Natriuresis was measured at baseline, at the end of the first treatment period, at the end of the placebo washout period, and at the end of the second treatment period. Patients were encouraged to increase or decrease salt intake with diet on the basis of the results of 24 h urinary sodium excretion. After randomisation, no change was introduced in diet micronutrients or macronutrients or caloric intake. No change in BP-lowering medications was allowed throughout the whole study period.

Patients' demographic characteristics and medication use were recorded at baseline assessment after completion of the placebo run-in period by survey. 24 h ambulatory BP was monitored at the start and end of each treatment period with TM-2430 equipment (A&D, Tokyo, Japan), which was set to obtain measurements at 15 min intervals during daytime (0600–2200 h) and 30 min intervals at night (2200–0600 h). All measurements were taken centrally at baseline and at each monthly visit to the CRC. The median of three measurements of albumin, sodium, and creatinine excretion, and UACR in 24 h urine collections was recorded for statistical analyses. Urine was collected for 3 consecutive days and the three collections handed over to investigators of the CRC at baseline assessment and each monthly visit. The coefficient of variation of 24 h albuminuria was 14·16% and of 24 h UACR was 13·88%. Glomerular filtration rate (GFR) was measured by iohexol plasma clearance.[15] Albumin and IgG fractional clearances were calculated by adjustments of albumin and IgG clearance for the simultaneously measured GFR.

We measured laboratory variables (calcium, phosphorus, glucose, HbA_{1c}, and potassium concentration, lipid profile, complete blood cell count, and creatinine concentration) at each assessment using standard techniques. We measured urinary albumin concentration by nephelometry, measured serum intact parathyroid hormone (iPTH) concentrations with the Access 2 Intact PTH assay (Beckman-Coulter, Milan, Italy), and measured 25-hydroxyvitamin D concentration with an automated direct competitive chemiluminescent immunoassay (ADVIA Centaur Vitamin D Total; Siemens, Milan, Italy). We recorded data locally in case report forms and then entered it twice into a central database held by the clinical research centre. Samples collected for possible explanatory analyses (for a-posteriori consideration to assess mechanisms that might mediate the albuminuria-lowering effect, if any, of study treatments) are listed in the appendix.

Outcomes

The primary efficacy outcome was the difference between the changes in 24 h albuminuria observed during the two 1 month treatment periods with paricalcitol or placebo, which was centrally assessed at the CRC. Secondary efficacy outcomes included 24 h UACR; 24 h BP recording; measured GFR; albumin and IgG fractional clearances; and adverse events. Other secondary outcome variables were 24 h 25-hydroxyvitamin D urinary concentration, serum calcium, phosphorus, and parathyroid hormone concentration; 24 h urinary calcium excretion; and serum total, LDL, and HDL cholesterol and triglyceride concentration. Further secondary outcomes that will be reported elsewhere are pulse wave velocity and other markers of vascular stiffness; plasma renin activity; plasma renin and pro-renin, angiotensin II, aldosterone, and brain natriuretic peptide concentrations; 24 h urinary aldosterone excretion; bone-specific alkaline phosphatase concentration; 24 h 25-hydroxyvitamin D urinary excretion; apolipoprotein A and B concentration; and serum C-reactive protein, 24 h urinary monocyte chemoattractant protein 1, transforming growth factor β, and RANTES excretion.

Changes in 24 h albuminuria, as well as in secondary outcomes, were assessed at 1 month, 2 months, and 3 months after randomisation to the diet groups as compared with baseline, and at the end of the two treatment periods with paricalcitol or placebo as compared with pretreatment values, considered independently of sodium diet group allocation as well as in the high-sodium and low-sodium diet groups separately.

We assessed safety by monitoring of vital signs, physical examination (BP and heart rate), laboratory tests, adverse event data, and documentation of additional medication use, assessed at baseline and at the end of each monthly visit. We summarised adverse events according to the Medical Dictionary for Regulatory Activities (version 18.0) organ system classification and preferred terms. Adverse events could be reported by patients at any time during the study, including the visits at baseline and the monthly visits up to study end.

Statistical analysis

On the basis of a previous study,[14] we hypothesised that patients would present with a mean 24 h albuminuria of 1033 mg (±516 mg) at baseline. We assumed that paricalcitol would reduce 24 h albuminuria by 40% (from 1033 mg to 620 mg) during high sodium intake and 10% (from 1033 mg to 930 mg) during low sodium

intake. We estimated an overall 10% albuminuria reduction during placebo therapy independent of sodium intake. Assuming a two-sided α of 0·05, 90 patients (45 on a high-sodium diet and 45 on a low-sodium diet) would be required to provide an 80% power to detect a significant antiproteinuric effect of paricalcitol versus placebo, irrespective of sodium intake. In the high-sodium group, 45 patients would provide 97% power to detect a within-group difference in the antiproteinuric effect of paricalcitol compared with placebo. To account for an expected 20% dropout, we aimed to include 112 patients.

We analysed the primary efficacy variable with a Wilcoxon signed-rank test and, with a multivariable approach, by using a linear mixed model for repeated measures (PROC MIXED; SAS version 9.2), with sequence, period, and treatment as fixed effects and participant as random effect. We interpreted a

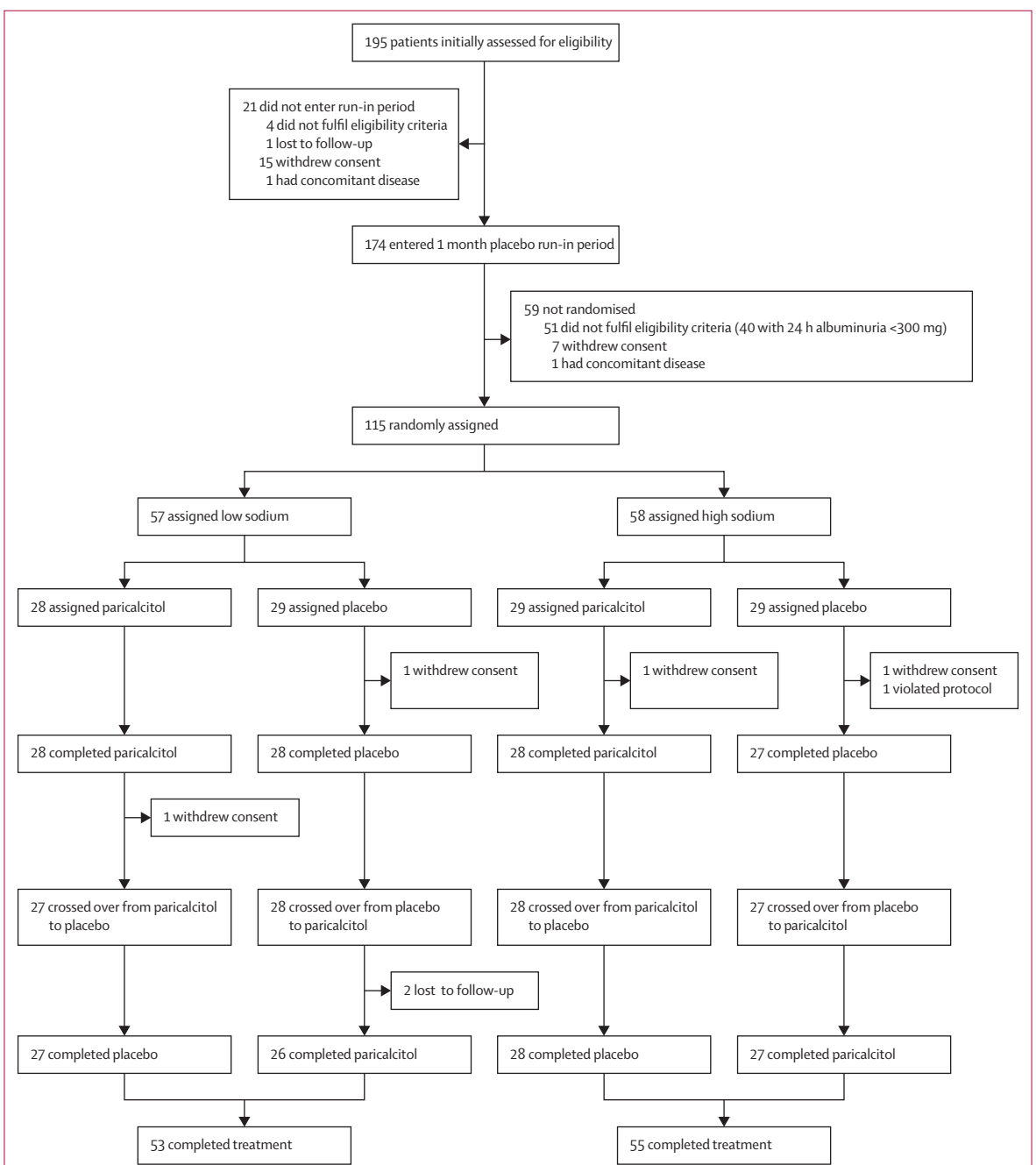

Figure 1: **Trial profile**

non-significant (p>0·05) effect of the treatment × sequence interaction as indicating absence of carryover effects; however, because of the low power of this test, this data interpretation should be taken with caution. We compared high and low sodium intake and all of the other between-group effects with ANCOVA, adjusted for baseline measurement. We did all remaining secondary and exploratory efficacy and safety assessments using paired *t* tests, Wilcoxon signed-rank tests, or McNemar tests (for within-group comparisons) and unpaired *t* tests, Wilcoxon rank-sum tests, χ^2 tests, or Fisher's Exact tests (for between-group comparisons), as appropriate.

To account for the possible confounding effect of early changes in natriuresis shortly after randomisation, we did sensitivity analyses by comparing the effect of paricalcitol and placebo during the first and the second study periods (before and after crossover) considered separately. According to the modified intention-to-treat principle, all efficacy and safety analyses considered all randomly assigned participants who took at least one dose of study drug and had an efficacy measurement at the end of the first treatment period, irrespective of protocol violations. We obtained baseline laboratory results after the run-in period. For multiple comparisons of albuminuria between high or low sodium intake, we set the significance level at 0·025 (Bonferroni's correction). We used SAS version 9.2 or Stata version 12 for all of the analyses. All p values were two-sided.

This study is registered with ClinicalTrials.gov, number NCT01393808, and the EU Clinical Trials Register, EudraCT number 2011-001713-14.

Role of the funding source

This was a sponsored, but fully independent trial. The funder freely supplied paricalcitol or placebo capsules and covered the costs of the study, but had no role in data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Oct 13, 2011, and Dec 18, 2014, we screened 195 patients (figure 1). 80 (41%) of these patients were not randomly allocated: 55 (69%) did not fulfil the eligibility criteria (including 40 [73%] with 24 h albuminuria of <300 mg) and 22 (28%) withdrew their consent, two (3%) had concomitant diseases, and one (1%) was lost to follow-up. Thus, between Dec 13, 2011, and Feb 17, 2015, we randomly allocated 115 patients: 57 (50%) to a low-sodium diet and 58 (50%) to a high-sodium diet. Within each diet group, we randomly allocated 57 (50%) patients to receive paricalcitol followed by placebo and 58 (50%) to receive placebo followed by paricalcitol. After randomisation, four (3%) patients withdrew consent, two (2%) were lost to follow-up, and one (1%) was excluded because of a protocol violation. All randomly allocated patients, however, received at least one dose of the study drug and were included in modified intention-to-treat analyses using the data up to the last study visit attended by each patient.

At randomisation, patient characteristics were similar between diet groups (appendix) and between paricalcitol-to-placebo or placebo-to-paricalcitol treatment groups (table 1). All patients but one (in the high-sodium group) had vitamin D deficiency or insufficiency at inclusion. All patients were taking the predefined 100 mg daily dose of losartan without ACE inhibitor therapy. The distribution of other medications was balanced between groups, except for some differences in the proportion of patients taking lipid-lowering agents (appendix).

At baseline, 24 h natriuresis was around 200 mEq in both sodium diet groups (table 1, figure 2). In the high-sodium diet group, it slightly increased during the study. In the low-sodium diet group, it significantly decreased by 1 month after randomisation and remained lower than at baseline throughout the whole observation period. Thus, changes in natriuresis at each timepoint as compared with baseline were significantly different between diet groups. At month 1, month 2, and month 3 after randomisation, natriuresis in the high-sodium diet group was about 200 mEq per day compared with 170 mEq per day in the low-sodium group. The difference between groups averaged at 35·2 mEq (SD 61·8) per day at month 1 (p=0·0003), 27·4 mEq (56·2) per day at month 2 (p=0·01), and 35·3 mEq (58·6) per day at month 3 (p=0·0002), even after adjustment for baseline sodium excretion. The salt intake reduction achieved at the end of the 3 month study period compared with baseline was 1·7–1·8 g.

24 h BP was similar between the two diet groups at baseline (appendix). At follow-up, systolic and diastolic BP did not change appreciably in the high-sodium diet group, whereas they slightly but significantly decreased in the low-sodium diet group. However, at each timepoint, BP never appreciably differed between the two diet groups, even after adjustment for baseline BP values. Bodyweight and HbA_{1c} concentration were stable in the high-sodium diet group and slightly but significantly decreased in the low-sodium diet group. Changes in HbA_{1c} concentration were significantly different between diet groups (appendix).

Albuminuria was similar between the two diet groups at baseline (table 2). Subsequently, albuminuria slightly and progressively increased up to month 3 in the high-sodium diet group, but did not change significantly from baseline. Conversely, in the low-sodium diet group, albuminuria significantly decreased from baseline to month 3 after randomisation. At each timepoint, percentage changes in albuminuria significantly differed between diet groups (table 2, figure 2). Differences in absolute changes were also significant, as were between-group differences in albuminuria, even after adjustment for baseline albuminuria by ANCOVA.

Multivariable regression analyses substantiated that albuminuria significantly differed between diet groups,

	Overall (n=115)	Low-sodium diet (n=57)		High-sodium diet (n=58)	
		Paricalcitol to placebo (n=28)	Placebo to paricalcitol (n=29)	Paricalcitol to placebo (n=29)	Placebo to paricalcitol (n=29)
Demographic characteristics					
Age (years)	64·4 (8·7)	65·0 (6·3)	64·2 (8·2)	64·2 (10·8)	64·1 (9·1)
Sex					
Male	102 (89%)	28 (100%)	26 (90%)	23 (79%)	25 (86%)
Female	13 (11%)	0	3 (10%)	6 (21%)	4 (14%)
Smoker					
Never	24 (21%)	6 (21%)	3 (10%)	4 (14%)	11 (38%)
Current	35 (30%)	7 (25%)	12 (41%)	10 (34%)	6 (21%)
Former	56 (49%)	15 (54%)	14 (48%)	15 (52%)	12 (41%)
BMI (kg/m^2)	30·8 (4·8)	31·2 (3·5)	31·4 (5·0)	29·1 (4·0)	31·7 (6·1)
Weight (kg)	88·0 (16·4)	90·7 (13·7)	91·5 (16·7)	81·1 (14·6)	88·7 (18·8)
24 h blood pressure					
Systolic (mm Hg)	146·3 (12·0)	147·6 (12·0)	148·2 (14·1)	146·3 (11·4)	143·1 (12·6)
Diastolic (mm Hg)	79·7 (6·0)	81·1 (5·4)	79·0 (7·0)	79·8 (5·9)	77·9 (5·2)
Mean (mm Hg)	101·6 (7·0)	102·9 (6·8)	102·5 (7·8)	101·6 (6·5)	99·3 (6·4)
Laboratory variables					
HbA_{1c} concentration (%)	7·5% (3·5)	7·7% (3·4)	7·4% (3·2)	7·8% (3·7)	7·0% (3·4)
Serum glucose concentration (mg/dL)	153·4 (53·1)	148·7 (41·4)	149·6 (47·9)	163·8 (65·0)	151·3 (56·3)
Total cholesterol concentration (mg/dL)	172·6 (39·1)	166·9 (48·3)	171·9 (32·7)	174·7 (40·7)	176·9 (34·4)
HDL cholesterol concentration (mg/dL)	43·5 (12·5)	42·5 (12·1)	41·0 (9·6)	47·5 (14·2)	43·0 (13·3)
LDL cholesterol concentration (mg/dL)	103·2 (32·7)	94·7 (37·2)	105·4 (32·0)	107·2 (36·2)	105·1 (24·4)
Triglyceride concentration (mg/dL)	163·8 (107·7)	177·7 (140·5)	164·6 (71·1)	134·8 (78·5)	178·5 (125·3)
Serum calcium concentration (mg/dL)	9·2 (0·3)	9·1 (0·3)	9·1 (0·4)	9·3 (0·3)	9·2 (0·3)
Serum phosphorus concentration (mg/dL)	3·4 (0·5)	3·3 (0·4)	3·3 (0·6)	3·5 (0·5)	3·4 (0·5)
Serum iPTH concentration (pg/mL)	47·5 (21·9)	50·0 (22·4)	50·0 (20·0)	42·2 (22·1)	48·2 (23·2)
25-hydroxyvitamin D concentration (ng/mL)	11·9 (5·8)	12·2 (5·7)	12·1 (6·4)	10·6 (4·6)	12·8 (6·6)
Serum potassium concentration (mEq/L)	4·1 (0·5)	4·0 (0·5)	4·2 (0·6)	4·2 (0·6)	4·1 (0·4)
Haemoglobin concentration (g/dL)	13·5 (1·5)	13·8 (1·4)	13·7 (1·5)	13·5 (1·5)	13·1 (1·8)
Kidney function variables					
GFR (mL/min per 1·73 m^2)	87·36 (29·19)	87·33 (21·14)	94·22 (33·08)	81·51 (26·27)	86·37 (34·51)
24 h urinary sodium excretion (mEq)	190·4 (152·3–227·0)	184·4 (157·4–229·6)	203·1 (173·5–223·4)	200·2 (151·3–226·5)	181·8 (144·4–223·5)
24 h urinary albumin excretion (mg)	724 (418–1233)	749 (402–1493)	724 (449–1198)	750 (505–1338)	658 (389–1058)
24 h urinary albumin-to-creatinine ratio (mg/g)	519·7 (271·5–771·1)	447·0 (355·9–1070·9)	548·5 (254·1–693·9)	604·3 (421·8–806·9)	458·5 (240·7–683·3)
24 h urinary calcium excretion (mg)	80·6 (43·9–135·9)	82·7 (43·9–123·3)	90·6 (41·1–149·7)	76·3 (45·0–116·2)	67·6 (55·0–136·2)
24 h urinary phosphorus excretion (mg)	698·1 (532·6–904·8)	617·7 (542·7–828·5)	763·1 (546·0–986·2)	634·3 (429·2–860·8)	779·3 (539·1–914·5)

Data are mean (SD) or median (IQR) for continuous variables and n (%) for dichotomous variables. iPTH=intact parathyroid hormone. GFR=glomerular filtration rate.

Table 1: **Baseline characteristics**

even after adjustment for predefined baseline variables (age, sex, centre, and 24 h systolic and diastolic BP), with or without adjustment for baseline HbA_{1c} concentration, and after adjustment for concomitant changes in 24 h BP, with or without adjustment for concomitant changes in HbA_{1c} concentration (appendix). Percentage changes in 24 h urinary sodium and albumin excretion were positively correlated and all of the correlations were highly significant (month 3 r=0·43; p<0·0001; appendix). We obtained similar findings from sensitivity analyses considering 24 h UACR (table 3, figure 2). GFR was similar between diet groups at baseline and follow-up (appendix). Albumin and IgG fractional clearances were also similar between groups at baseline, and their changes with follow-up paralleled those of albuminuria (appendix, figure 2). Data for markers of mineral metabolism are shown in the appendix.

In the study group considered as a whole, albuminuria significantly decreased with paricalcitol over the 1 month treatment period, whereas it did not change appreciably with placebo (table 2, figure 3). Percentage changes between treatment periods were significantly different. We observed similar changes by assessing absolute changes (table 2) and 24 h UACR (table 3). Consistently, albumin fractional clearance significantly decreased with paricalcitol, whereas changes in placebo were not

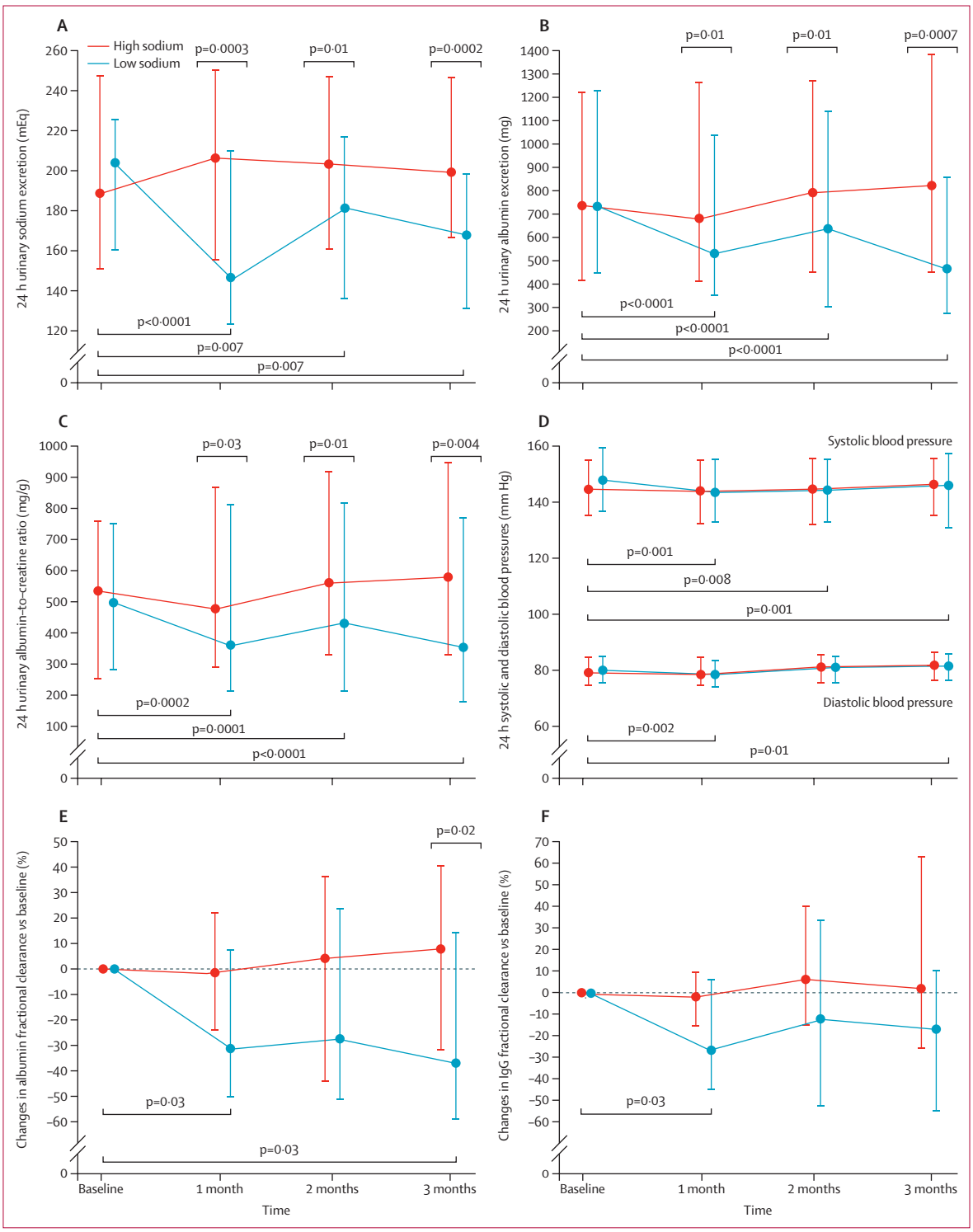

Figure 2: **Changes in urinary sodium excretion, kidney function variables, and blood pressure control during the study period, according to sodium diet group**
Median 24 h urinary sodium (A) and albumin (B) excretion and albumin-to-creatinine ratio (C) and mean systolic and diastolic blood pressure (D) and median percentage changes in albumin (E) and IgG (F) fractional clearances versus baseline in high-sodium and low-sodium intake groups. Datapoints are medians with IQR error bars or means with SD error bars. p values for intragroup changes are shown at the bottom and for differences in changes between groups at the top. Intragroup changes in the high-sodium diet group were not significant. Only significant p values are shown.

significant (appendix). IgG fractional clearances, 24 h systolic and diastolic BP, and metabolic variables did not change appreciably from the beginning to the end of the two 1 month treatment periods, whereas we observed a small but significant decrease in GFR with placebo (appendix). Serum calcium, phosphorus, and 25-hydroxyvitamin D concentrations significantly increased and serum iPTH concentrations significantly decreased with paricalcitol treatment. 24 h urinary calcium excretion more than doubled with paricalcitol and did not change appreciably with placebo, whereas 24 h urinary phosphorus excretion increased with paricalcitol but did not change with placebo, but changes were significantly different between study drugs.

In the low-sodium diet group, albuminuria decreased during both treatment periods similarly with paricalcitol and placebo (table 2, figure 3, appendix). Notably, however, in the high-sodium diet group, albuminuria showed a different trend, decreasing with paricalcitol and not significantly changing with placebo, but changes were significantly different between diet groups. Percentage changes in albuminuria significantly differed between 1 month treatment periods in the high-sodium group (table 2, figure 3). In the low-sodium diet group, the absolute reduction in albuminuria was significant during both treatment periods and changes were similar between paricalcitol and placebo (table 2). However, in the high-sodium diet group, the absolute reduction in albuminuria was significant only during paricalcitol treatment. Percentage changes in albuminuria significantly differed between diet groups in patients taking placebo (figure 3). Changes in 24 h UACR were similar to changes observed in 24 h albuminuria (table 3). With high sodium intake, IgG fractional clearance significantly increased with placebo but not with paricalcitol (figure 3, appendix). Percentage changes in IgG fractional clearances significantly differed between paricalcitol and placebo in the high-sodium group.

Albuminuria significantly decreased independent of sodium intake when patients were on paricalcitol treatment. 24 h UACR and albumin fractional clearance also significantly decreased (table 2, 3, figure 3, appendix). Percentage changes in 24 h albuminuria and albumin and IgG fractional clearances significantly differed between low-sodium and high-sodium diet groups when patients were on placebo. BP decreased with the low-sodium diet

	Baseline (mg [IQR])	After treatment (mg [IQR])	Absolute change (mg [95% CI])	p value	Percentage change (% [95% CI])	p value
Low-sodium or high-sodium diet						
Baseline to month 1						
Low	724 (441 to 1233)	519 (325 to 1080)	−144·0 (−217·4 to −102·2)	<0·0001	−31·5% (−37·6 to −15·7)	<0·0001
High	730 (416 to 1227)	672 (403 to 1290)	−49·0 (−201·6 to 21·6)	0·38	−10·0% (−20·9 to 3·2)	0·53
Low *vs* high	..	..	−121·0 (−259·2 to −1·4)	0·048	−19·6% (−34·3 to −6·7)	0·005
Baseline to month 2						
Low	724 (441 to 1233)	626 (292 to 1127)	−106·6 (−181·4 to −69·1)	0·0002	−19·4% (−27·4 to −11·6)	<0·0001
High	730 (416 to 1227)	783 (468 to 1295)	−13·0 (−115·2 to 116·6)	0·85	−0·7% (−17·9 to 23·9)	0·41
Low *vs* high	..	..	−152·6 (−279·4 to −18·0)	0·02	−21·3% (−38·7 to −6·2)	0·006
Baseline to month 3						
Low	724 (441 to 1233)	481 (289 to 837)	−237·6 (−385·9 to −151·2)	<0·0001	−36·6% (−44·9 to −28·5)	<0·0001
High	730 (416 to 1227)	801 (441 to 1365)	20·2 (−128·2 to 93·6)	0·63	2·9% (−16·8 to 16·4)	0·48
Low *vs* high	..	..	−288·0 (−463·7 to −138·2)	0·0005	−32·4% (−48·8 to −17·2)	<0·0001
Paricalcitol or placebo treatment*						
Overall						
Paricalcitol	711 (413 to 1225)	589 (338 to 1077)	−108·0 (−145·4 to −63·4)	<0·0001	−16·5% (−26·9 to −9·2)	0·002
Placebo	760 (415 to 1227)	667 (364 to 1243)	−40·3 (−97·9 to 10·1)	0·12	−4·9% (−14·9 to 3·0)	0·27
Paricalcitol *vs* placebo	..	..	−74·9 (−125·3 to −15·8)	0·09	−12·5% (−20·3 to −3·0)	0·04
Low sodium						
Paricalcitol	689 (356 to 1225)	540 (289 to 981)	−108·0 (−145·4 to −60·5)	0·0005	−17·7% (−34·2 to −4·4)	0·006
Placebo	724 (415 to 1198)	481 (315 to 1084)	−102·2 (−198·7 to 46·1)	0·001	−20·1% (−31·7 to −4·5)	0·0002
Paricalcitol *vs* placebo	..	..	−33·1 (−128·2 to 56·2)	0·55	−4·1% (−21·6 to 9·3)	0·59
High sodium						
Paricalcitol	750 (505 to 1316)	651 (403 to 1120)	−108·0 (−201·6 to −41·8)	0·049	−14·7% (−23·9 to −4·7)	0·10
Placebo	789 (408 to 1287)	831 (487 to 1365)	23·0 (−66·2 to 66·2)	0·43	3·7% (−9·0 to 12·7)	0·10
Paricalcitol *vs* placebo	..	..	−108·0 (−273·6 to −24·5)	0·09	−17·8% (−32·3 to −3·9)	0·02

Data are median (IQR) or median (95% CI). *Baseline is at the start of treatment and after treatment is after the 1 month of paricalcitol or placebo treatment.

Table 2: **24 h urinary albumin excretion**

	Baseline (mg/g [IQR])	After treatment (mg/g [IQR])	Absolute change (mg/g [95% CI])	p value	Percentage change (% [95% CI])	p value
Low-sodium or high-sodium diet						
Baseline to month 1						
Low	502 (288 to 762)	388 (212 to 826)	−115·9 (−155·3 to −65·3)	0·0001	−24·5% (−36·6 to −17·0)	0·0002
High	544 (272 to 771)	492 (294 to 874)	−8·5 (−100·7 to 19·5)	0·61	−2·9% (−19·1 to 7·2)	0·84
Low *vs* high	..	..	−99·4 (−192·8 to −12·0)	0·03	−20·1% (−34·4 to −6·4)	0·007
Baseline to month 2						
Low	502 (288 to 762)	408 (209 to 835)	−86·0 (−142·5 to −59·2)	<0·0001	−22·0% (−30·4 to −13·6)	0·0001
High	544 (272 to 771)	572 (307 to 908)	2·1 (−72·6 to 94·6)	0·46	1·2% (−13·0 to 16·9)	0·25
Low *vs* high	..	..	−126·9 (−218·6 to −43·7)	0·003	−24·9% (−38·6 to −10·5)	0·0008
Baseline to month 3						
Low	502 (288 to 762)	359 (187 to 721)	−112·5 (−258·5 to −66·5)	<0·0001	−30·8% (−43·9 to −19·9)	<0·0001
High	544 (272 to 771)	575 (330 to 919)	15·6 (−48·0 to 92·4)	0·33	5·8% (−7·6 to 18·6)	0·22
Low *vs* high	..	..	−185·6 (−302·6 to −91·4)	0·0005	−32·4% (−48·0 to −16·9)	0·0002
Paricalcitol or placebo treatment*						
Overall						
Paricalcitol	489 (271 to 853)	427 (227 to 721)	−68·6 (−121·1 to −32·2)	0·0007	−18·2% (−22·9 to −10·1)	0·005
Placebo	486 (255 to 895)	461 (259 to 887)	5·0 (−47·0 to 31·5)	0·58	1·5% (−9·3 to 8·8)	0·76
Paricalcitol *vs* placebo	..	..	−31·1 (−117·6 to 5·3)	0·11	−6·7% (−20·1 to 0·7)	0·06
Low sodium						
Paricalcitol	397 (242 to 967)	386 (187 to 717)	−67·8 (−128·2 to −32·2)	0·003	−17·5% (−28·2 to −11·7)	0·01
Placebo	453 (255 to 762)	365 (209 to 788)	−49·1 (−88·3 to 3·6)	0·03	−12·2% (−24·9 to 1·7)	0·005
Paricalcitol *vs* placebo	..	..	−7·5 (−133·3 to 27·6)	0·62	−4·4% (−18·2 to 6·0)	0·65
High sodium						
Paricalcitol	588 (282 to 853)	464 (286 to 771)	−89·7 (−133·7 to −6·8)	0·06	−18·7% (−23·4 to −0·9)	0·14
Placebo	488 (254 to 1128)	575 (343 to 959)	33·1 (−5·8 to 82·9)	0·21	10·6% (−1·7 to 19·3)	0·03
Paricalcitol *vs* placebo	..	..	−91·1 (−171·1 to 30·8)	0·10	−14·4% (−32·7 to −1·1)	0·03

Data are median (IQR) or median (95% CI). *Baseline is at the start of treatment and after treatment is after the 1 month of paricalcitol or placebo treatment.

Table 3: **24 h urinary albumin-to-creatinine ratio**

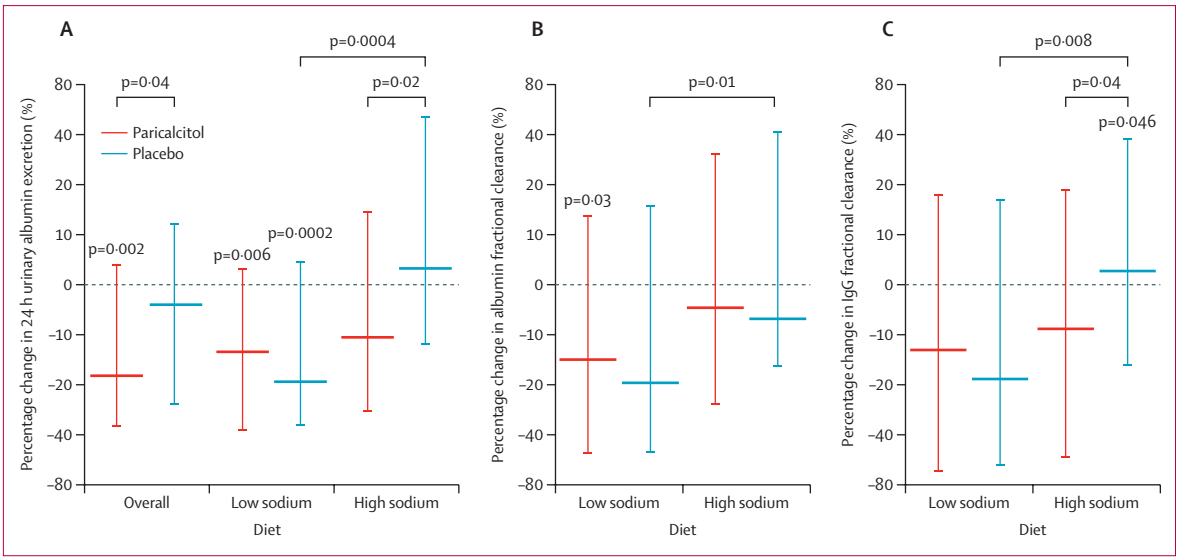

Figure 3: **Kidney sieving function according to sodium diet and treatment period**
Percentage changes during paricalcitol or placebo treatment in 24 h urinary albumin excretion (A) and in albumin (B) and IgG (C) fractional clearances. Datapoints are medians with IQR error bars. p values for intragroup changes are shown above the error bars and for differences in changes between groups at the top. Only significant p values are shown.

during both 1 month treatment periods during paricalcitol therapy, and did not change appreciably in patients taking either paricalcitol or placebo with the high-sodium diet (appendix). Changes in other considered variables are summarised in the appendix. With the low-sodium diet, albuminuria significantly decreased with both paricalcitol and placebo during the first period after randomisation (appendix). Changes in albuminuria significantly differed between the two diet groups during placebo treatment, but not during paricalcitol therapy. During the second treatment period, with patients on stable diet, albuminuria did not significantly change with placebo or paricalcitol in both diet groups.

No patient left the study because of side-effects. One patient had a stroke and one had a cardiac ischaemic event during paricalcitol therapy. We observed 65 non-serious adverse events in 51 (44%) patients during paricalcitol treatment and 44 events in 36 (31%) patients during placebo treatment (table 4). Among possibly paricalcitol-related effects, we observed 14 cases of hypercalciuria, six cases of hypercalcaemia, and five cases of hyperphosphataemia in one patient each. We observed one case of hypercalciuria in one (1%) patient during placebo treatment. We also observed 11 cases of hypocalciuria in 11 (10%) patients during the placebo treatment period. All of these events were asymptomatic. No patients died during the study.

	Overall		Paricalcitol		Placebo	
	Patients (n=115)	Events	Patients (n=115)	Events	Patients (n=115)	Events
All adverse events						
Total	66 (57%)	111	52 (45%)	67	36 (31%)	44
Serious adverse events*						
Ischaemic heart disease	1 (1%)	1	1 (1%)	1	0	0
Stroke	1 (1%)	1	1 (1%)	1	0	0
Total	2 (2%)	2	2 (2%)	2	0	0
Non-serious adverse events†						
Hypercalciuria	14 (12%)	15	14 (12%)	14	1 (1%)	1
Hypocalciuria	14 (12%)	14	3 (3%)	3	11 (10%)	11
Flu-like symptoms, cough, bronchitis, or synusitis	9 (8%)	9	7 (6%)	7	2 (2%)	2
Hyperphosphaturia	7 (6%)	8	2 (2%)	2	6 (5%)	6
Hypercalcaemia	6 (5%)	6	6 (5%)	6	0	0
Hyperphosphataemia	5 (4%)	5	5 (4%)	5	0	0
Hypophosphaturia	4 (3%)	4	2 (2%)	2	2 (2%)	2
Low serum iPTH concentration	3 (3%)	3	3 (3%)	3	0	0
Gastroenteritis, diarrhoea, or abdominal pain	3 (3%)	3	2 (2%)	2	1 (1%)	1
Headache or migraine	3 (3%)	3	2 (2%)	2	1 (1%)	1
Hypokalaemia, hyperhomocysteinaemia, or low bone alkaline phosphatase concentration	3 (3%)	3	2 (2%)	2	1 (1%)	1
Urinary tract infection or macrohaematuria	3 (3%)	3	1 (<1%)	1	2 (2%)	2
Thrombocytopenia or anaemia	3 (3%)	3	1 (1%)	1	2 (2%)	2
Tinnitus, hands tingling, or dizziness	3 (3%)	3	1 (1%)	1	2 (2%)	2
Fever or asthenia (unspecified)	2 (2%)	2	2 (2%)	2	0	0
Lumbar pain or cramps	2 (2%)	2	2 (2%)	2	0	0
Dermatitis and skin infection	2 (2%)	2	2 (2%)	2	0	0
Osteoarthritis and trigger finger	1 (1%)	2	1 (1%)	2	0	0
Traumatic pain or tendon tear	2 (2%)	2	1 (1%)	1	1 (1%)	1
Allergic reactions, urticaria, or pruritus	2 (2%)	2	1 (1%)	1	1 (1%)	1
Hypotension	2 (2%)	2	0	0	2 (2%)	2
Hypocalcaemia	2 (2%)	2	0	0	2 (2%)	2
Hypophosphataemia	2 (2%)	2	0	0	2 (2%)	2
Herpes zoster infection	1 (1%)	1	1 (1%)	1	0	0
Legs oedema	1 (1%)	1	1 (1%)	1	0	0
Worsening symptoms of ulcerous colitis	1 (1%)	1	1 (1%)	1	0	0
Bilateral cataract	1 (1%)	1	1 (1%)	1	0	0
Complete right bundle branch block	1 (1%)	1	0	0	1 (1%)	1
Complete left bundle branch block	1 (1%)	1	0	0	1 (1%)	1
Ventricular extrasystoles	1 (1%)	1	0	0	1 (1%)	1
Deep vein thrombosis	1 (1%)	1	0	0	1 (1%)	1
Transient increase in serum creatinine concentration	1 (1%)	1	0	0	1 (1%)	1
Total	66 (57%)	109	51 (44%)	65	36 (31%)	44

Normal ranges: serum calcium concentration: 8·7–10·3 mg/dL, serum phosphate concentration: 2·3–4·7 mg/dL, urinary calcium excretion: 100–300 mg over 24 h, urinary phosphate excretion: 300–1000 mg over 24 h. iPTH=intact parathyroid hormone. *Both events grade 4. †All events grade 1–2.

Table 4: **Adverse events**

Discussion

We found that a low-sodium diet promptly and persistently reduced albuminuria independently of concomitant treatment with paricalcitol or placebo in patients with type 2 diabetes with residual macroalbuminuria despite full-dose losartan therapy. Paricalcitol significantly reduced residual albuminuria compared with placebo in both diet groups combined. This effect, however, was fully driven by the protective effect of paricalcitol against the sodium-induced increase in albuminuria that was observed during placebo when patients were on the high-sodium diet, whereas paricalcitol induced no difference in albuminuria versus placebo in patients on the low-sodium diet. Thus, salt intake restriction might achieve a persistent and clinically relevant albuminuria-lowering effect, even in patients with residual macroalbuminuria despite optimal guideline-directed therapy[7] that includes full-dose losartan. This finding could explain the retrospective evidence that low salt intake is associated with an improvement in the nephroprotective and cardioprotective effect of ARB therapy in patients with type 2 diabetes with overt nepropathy.[8] Of note, albuminuria decreased even with moderate salt restriction, averaging approximately 1·7–1·8 g per day (equivalent to 30 mEq of sodium per day). This observation might have clinically relevant implications since patient adherence to dietary guidelines is the major barrier to their effectiveness.[16] Thus, in everyday clinical practice, patients might be more compliant with a moderate sodium restriction that only

marginally affects food palatability than with much more restrictive diets that are similarly effective on albuminuria[10] but poorly tolerated by most patients. In this context, paricalcitol might help to prevent further increases in albuminuria caused by excess salt intake in patients with diabetes and macroalbuminuria who are poorly compliant with diet recommendations.

After randomisation, no change was introduced in diet micronutrients or macronutrients or caloric intake. Moreover, the dose of losartan was fixed in all patients and no change in BP-lowering medications was allowed throughout the whole study period. Thus, we could determine that albuminuria reduction was a direct effect of salt restriction, and the slight bodyweight reduction observed in patients allocated to the low-sodium diet at least in part reflected a reduction in fluid volume.[10] Changes in albuminuria strongly correlated with concomitant changes in sodium excretion. These findings—combined with evidence that measured GFR was similar in both diet groups throughout the whole study period, whereas changes in albumin and IgG fractional clearances closely paralleled those in albuminuria—suggest that moderate salt restriction might directly improve the glomerular sieving function without appreciably affecting glomerular filtration.

BP was only marginally affected by sodium intake, and sodium-related changes in albuminuria were significant even with multivariable analyses adjusting for concomitant fluctuations in 24 h BP profiles. Thus, by contrast with previous studies[10] that found an association between albuminuria and BP reduction, our findings suggest that the reduction in albuminuria achieved through salt restriction is at least in part independent of systemic BP. As frequently observed in patients with diabetic and non-diabetic chronic kidney disease,[8,17] as well as in the general population worldwide,[18] salt intake at inclusion was remarkably high in our study patients. Thus, salt restriction achieved in the low-salt diet group resulted in sodium intake that was still considerably more than the recommended intake of 85 mEq of sodium per day.[19]

HbA_{1c} concentrations decreased in patients on the low-sodium diet as compared with those without salt intake restriction. Even if no changes in diet and physical activity were intentionally introduced during the study, the close patient–dietitian interactions intended to achieve the salt intake goals might also have resulted in improved compliance with other diet recommendations, or the reduced food palatability caused by even moderate salt restriction might have led to reduced calorie intake and weight loss. This hypothesis is intriguing since caloric restriction is associated with amelioration of glomerular hyperfiltration,[20] one of the strongest risk factors for renal disease onset and progression in patients with diabetes.[21] Thus, at least in theory, salt restriction might have an additional, albeit indirect, clinical benefit associated with reduced calorie intake. However, multivariable analyses adjusting for changes in HbA_{1c} concentration during follow-up substantiated that the effect of salt restriction on albuminuria was not appreciably affected by improved glycaemia.

We also found that 1 month treatment with paricalcitol significantly reduced albuminuria compared with baseline and with placebo. These findings concur with data from patients with macroalbuminuria from the VITAL trial[14] showing that 24 week treatment with 2 µg per day of paricalcitol decreased mean 24 h albuminuria as compared with placebo in patients with type 2 diabetes on stable losartan therapy.

Whereas in VITAL, the albuminuria-lowering effect was at least in part explained by a concomitant treatment-induced BP reduction, the treatment effect in our study was independent of 24 h BP control, which was identical during paricalcitol and placebo treatment. GFR, which was not measured in VITAL, was also similar during both treatment periods. These data, combined with evidence that in the high-sodium diet group, paricalcitol prevented the increase in IgG fractional clearance that was observed during the placebo treatment period, strongly suggest that the protective effect of paricalcitol against a salt-induced increase in albuminuria can be mediated by a direct effect on the glomerular barrier sieving function. However, the finding that albuminuria similarly decreased during the two treatment periods in patients randomly allocated to low sodium intake showed that the effect of paricalcitol on albuminuria and glomerular sieving function is salt dependent. Notably, the albuminuria-lowering effect of moderate salt restriction was persistent over time.

These findings suggest that evidence from the ViRTUE-CKD clinical trial,[22] which showed that paricalcitol has no additional albuminuria-lowering effects independent of sodium intake in patients with chronic kidney disease but without diabetes on single-agent RAS blockade, does not apply to patients with type 2 diabetes. Indeed, paricalcitol might have a specific role in nephroprotection in patients with diabetic renal disease and poor compliance with low-sodium-intake dietary recommendations, possibly because sodium overload increases intrarenal ACE activity, which enhances conversion of angiotensin I to angiotensin II and blunts the effects of RAS inhibition in rats and human beings with high sodium intake.[23] Angiotensin II might directly increase the glomerular barrier permeability to plasma macromolecules and proteinuria, independent of changes in systemic haemodynamics and even intraglomerular capillary presssure.[24] Independent of BP control, intrarenal ACE activation is associated with accelerated progression of experimental chronic kidney disease[2,4] and enhanced proteinuria and faster loss of renal function in patients with diabetic and non-diabetic chronic nephropathies.[8,17] However, activation of the vitamin D receptor suppresses renin transcription,[25] so vitamin D analogues might act as negative endocrine regulators of the RAS, in particular in

combination with ARBs,[11] as in our study population. This hypothesis could explain the reduction in proteinuria observed in patients with renal insufficiency receiving vitamin D supplementation to treat chronic kidney disease-related abnormalities in mineral-bone disease.[22,26] Thus, we speculate that in patients allocated to high-sodium intake, paricalcitol compensated for the reduced albuminuria-lowering effect of losartan.

1 month oral paricalcitol therapy was well tolerated and no patient stopped paricalcitol therapy because of side-effects. However, two serious adverse events were reported, both cardiovascular, during paricalcitol treatment. 65 (60%) of the 109 non-serious adverse events were reported during paricalcitol treatment, and hypercalciuria, hypercalcaemia, and hyperphosphataemia were more frequent during treatment with paricalcitol than with placebo. Consistently, we observed a paricalcitol-induced increase in serum calcium and phosphate concentrations and a decrease in serum iPTH concentrations, which were independent of sodium intake and associated with an increase in urinary phosphate and in particular calcium excretion. These findings could have clinical implications since increased serum phosphate concentrations have been reported to attenuate the renoprotective effect of ACE inhibition in patients with non-diabetic proteinuric chronic kidney disease.[27] Although studies[14,26] of paricalcitol with long follow-up did not suggest major safety signals, our study was too short to establish a full safety profile of paricalcitol therapy in this context, and long-term studies are needed to weigh the benefits of blunted sodium-dependent changes in albuminuria versus the potential unfavourable effects of calcium phosphate tissue and urine precipitation or chronically suppressed PTH production.

Consistent with data from VITAL,[14] all patients but one had vitamin D deficiency or insufficiency at inclusion. Thus, further trials are needed to assess whether the treatment effect on albuminuria is related to a specific antifibrotic, nephroprotective effect as compared with calcitriol in addition to RAS inhibitor therapy,[28] which could be generalised to all patients with type 2 diabetes independent of vitamin D concentrations, or whether it is related to correction of vitamin D deficiency or insufficiency and could therefore also be obtained by supplementation of native vitamin D.[29] We did not measure markers of RAS activity and other possible mediators of proteinuria that could be affected by paricalcitol, such as transforming growth factor β or fibroblast growth factor 23, and used albuminuria as a surrogate marker of renal disease progression.

The difference in sodium excretion achieved between the two sodium diet groups was smaller than expected. Of note, all of our patients were from northern Italy, where diets are typically rich in meat and cheese (and salt), more similar to diets typically in Europe or North America than from the Mediterranean area. Thus, our findings might be generalisable to most patients from high-income and middle-income countries.

We estimated the number of participants a priori on the basis of the expected treatment effect, which made adequate power analyses possible despite the small sample size. Allocation to pharmacological treatment was randomised, double-blind, and placebo controlled. Data assessors were also masked to patient diet. Moreover, we monitored salt intake with the objective variable of 24 h urinary sodium excretion. We measured natriuresis in three consecutive 24 h urine collections and, as for 24 h albuminuria, we recorded the median of the measurements to reduce the noise of physiological fluctuations in salt intake and albumin excretion and the inherent imprecision of urine collections.[30] This methodological precaution, along with use of gold standard techniques to monitor BP and kidney function with ambulatory 24 h BP recording and direct GFR measurements, and the crossover design with patients serving as their own internal controls, reduced random data fluctuations and increased the statistical power of study analyses. The similarity of the 24 h albuminuria and UACR coefficients of variations reflected the precision of urine collections. The consistency between results of the primary outcome analyses and sensitivity analyses considering 24 h UACR substantiated the robustness of the findings. Another strength was the stable fixed-dose background losartan treatment. Finally, despite the technically challenging and labour-intensive design, the study had a high retention rate of enrolled participants and good adherence to the study interventions.

Even moderate salt intake restriction, averaging 30 mEq of sodium or 1·7–1·8 g of salt per day, is expected to achieve substantial nephroprotection in patients with type 2 diabetes at high renal and cardiovascular risk because of residual macroalbuminuria despite optimised conservative therapy. Avoidance of overly restrictive sodium targets could lead to increased food palatability and therefore improved patient compliance, with larger clinical benefit than that seen in populations who are poorly compliant with more restrictive guidelines. Prospective clinical trials, however, are needed to test the long-term risk-benefit profile of paricalcitol add-on therapy, particularly in the substantial proportion of patients with diabetes who are refractory to any dietary recommendation.

Contributors

PR and GR had the original idea, wrote the study protocol, coordinated the study centres, and critically revised the report. APa, MT, IPI, BR, MA, ACB, RT, and AB selected, monitored, and cared for patients and collected data. APe and FP did the statistical analysis. OD and NR monitored the study. FG, FC, and NS executed and interpreted centralised laboratory measurements. APa, MT, MAP, and PR analysed and interpreted data. MdB interpreted data and wrote the first draft of the report with the Scientific Writing Academy attendees. MAP revised the first draft and PR wrote the final version.

Declaration of interests

We declare no competing interests.

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References

1 de Boer IH, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J. Temporal trends in the prevalence of diabetic kidney disease in the United States. *JAMA* 2011; **305:** 2532–39.

2 Ruggenenti P, Cravedi P, Remuzzi G. Mechanisms and treatment of CKD. *J Am Soc Nephrol* 2012; **23:** 1917–28.

3 Remuzzi G, Schieppati A, Ruggenenti P. Clinical practice. Nephropathy in patients with type 2 diabetes. *N Engl J Med* 2002; **346:** 1145–51.

4 Ruggenenti P, Cravedi P, Remuzzi G. The RAAS in the pathogenesis and treatment of diabetic nephropathy. *Nat Rev Nephrol* 2010; **6:** 319–30.

5 Ruggenenti P, Perna A, Remuzzi G. Retarding progression of chronic renal disease: the neglected issue of residual proteinuria. *Kidney Int* 2003; **63:** 2254–61.

6 Holtkamp FA, de Zeeuw D, de Graeff PA, et al. Albuminuria and blood pressure, independent targets for cardioprotective therapy in patients with diabetes and nephropathy: a post hoc analysis of the combined RENAAL and IDNT trials. *Eur Heart J* 2011; **32:** 1493–99.

7 American Diabetes Association. Standards of medical care in diabetes—2011. *Diabetes Care* 2011; **34** (suppl 1)**:** S11–61.

8 Lambers Heerspink HJ, Holtkamp FA, Parving HH, et al. Moderation of dietary sodium potentiates the renal and cardiovascular protective effects of angiotensin receptor blockers. *Kidney Int* 2012; **82:** 330–37.

9 Suckling RJ, He FJ, Macgregor GA. Altered dietary salt intake for preventing and treating diabetic kidney disease. *Cochrane Database Syst Rev* 2010; **12:** CD006763.

10 Kwakernaak AJ, Krikken JA, Binnenmars SH, et al. Effects of sodium restriction and hydrochlorothiazide on RAAS blockade efficacy in diabetic nephropathy: a randomised clinical trial. *Lancet Diabetes Endocrinol* 2014; **2:** 385–95.

11 Zhang Z, Zhang Y, Ning G, Deb DK, Kong J, Li YC. Combination therapy with AT1 blocker and vitamin D analog markedly ameliorates diabetic nephropathy: blockade of compensatory renin increase. *Proc Natl Acad Sci USA* 2008; **105:** 15896–901.

12 Doorenbos CR, van den Born J, Navis G, de Borst MH. Possible renoprotection by vitamin D in chronic renal disease: beyond mineral metabolism. *Nat Rev Nephrol* 2009; **5:** 691–700.

13 Joergensen C, Tarnow L, Goetze JP, Rossing P. Vitamin D analogue therapy, cardiovascular risk and kidney function in people with type 1 diabetes mellitus and diabetic nephropathy: a randomized trial. *Diabet Med* 2015; **32:** 374–81.

14 de Zeeuw D, Agarwal R, Amdahl M, et al. Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): a randomised controlled trial. *Lancet* 2010; **376:** 1543–51.

15 Gaspari F, Perico N, Ruggenenti P, et al. Plasma clearance of nonradioactive iohexol as a measure of glomerular filtration rate. *J Am Soc Nephrol* 1995; **6:** 257–63.

16 Welch JL, Bennett SJ, Delp RL, Agarwal R. Benefits of and barriers to dietary sodium adherence. *West J Nurs Res* 2006; **28:** 162–80.

17 Vegter S, Perna A, Postma MJ, Navis G, Remuzzi G, Ruggenenti P. Sodium intake, ACE inhibition, and progression to ESRD. *J Am Soc Nephrol* 2012; **23:** 165–73.

18 Powles J, Fahimi S, Micha R, et al. Global, regional and national sodium intakes in 1990 and 2010: a systematic analysis of 24 h urinary sodium excretion and dietary surveys worldwide. *BMJ Open* 2013; **3:** e003733.

19 WHO. Guideline: sodium intake for adults and children. Geneva: World Health Organization, 2012.

20 Ruggenenti P, Abbate M, Ruggiero B, et al. Renal and systemic effects of calorie restriction in patients with type 2 diabetes with abdominal obesity: a randomized controlled trial. *Diabetes* 2017; **66:** 75–86.

21 Ruggenenti P, Porrini EL, Gaspari F, et al. Glomerular hyperfiltration and renal disease progression in type 2 diabetes. *Diabetes Care* 2012; **35:** 2061–68.

22 Keyzer CA, van Breda GF, Vervloet MG, et al. Effects of vitamin D receptor activation and dietary sodium restriction on residual albuminuria in CKD: the ViRTUE-CKD trial. *J Am Soc Nephrol* 2017; **28:** 1296–305.

23 Krikken JA, Laverman GD, Navis G. Benefits of dietary sodium restriction in the management of chronic kidney disease. *Curr Opin Nephrol Hypertens* 2009; **18:** 531–38.

24 Lapinski R, Perico N, Remuzzi A, Sangalli F, Benigni A, Remuzzi G. Angiotensin II modulates glomerular capillary permselectivity in rat isolated perfused kidney. *J Am Soc Nephrol* 1996; **7:** 653–60.

25 Freundlich M, Quiroz Y, Zhang Z, et al. Suppression of renin-angiotensin gene expression in the kidney by paricalcitol. *Kidney Int* 2008; **74:** 1394–402.

26 de Borst MH, Hajhosseiny R, Tamez H, Wenger J, Thadhani R, Goldsmith DJ. Active vitamin D treatment for reduction of residual proteinuria: a systematic review. *J Am Soc Nephrol* 2013; **24:** 1863–71.

27 Zoccali C, Ruggenenti P, Perna A, et al. Phosphate may promote CKD progression and attenuate renoprotective effect of ACE inhibition. *J Am Soc Nephrol* 2011; **22:** 1923–30.

28 Rubel D, Stock J, Ciner A, et al. Antifibrotic, nephroprotective effects of paricalcitol versus calcitriol on top of ACE-inhibitor therapy in the *COL4A3* knockout mouse model for progressive renal fibrosis. *Nephrol Dial Transplant* 2014; **29:** 1012–19.

29 Susantitaphong P, Nakwan S, Peerapornratana S, et al. A double-blind, randomized, placebo-controlled trial of combined calcitriol and ergocalciferol versus ergocalciferol alone in chronic kidney disease with proteinuria. *BMC Nephrol* 2017; **18:** 19.

30 Olde Engberink RH, van den Hoek TC, van Noordenne ND, van den Born BH, Peters-Sengers H, Vogt L. Use of a single baseline versus multiyear 24-hour urine collection for estimation of long-term sodium intake and associated cardiovascular and renal risk. *Circulation* 2017; **136:** 917–26.