Effects of Sevelamer Carbonate in Patients With CKD and Proteinuria: The ANSWER Randomized Trial

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Rationale & Objective: Hyperphosphatemia is associated with increased risk for chronic kidney disease (CKD) progression and reduced antiproteinuric effects of renin-angiotensin system (RAS) blockers. We investigated whether the phosphate binder sevelamer carbonate may enhance the antiproteinuric effect of RAS inhibitors in patients with CKD.

Study Design: Phase 2, randomized, controlled, open-label, crossover trial.

Setting & Participants: Between November 2013 and December 2014, we enrolled 53 patients with CKD with estimated glomerular filtration rates (eGFRs) > 15 mL/min/1.73 m^2 and residual proteinuria with protein excretion ≥ 0.5 g/24 h despite maximal tolerated ramipril and/or irbesartan therapy from 2 nephrology units in Italy.

Intervention: After stratification by serum phosphate level, ≤4 or >4 mg/dL, patients were randomly assigned to 3 months of sevelamer (1,600 mg thrice daily) treatment followed by 3 months without sevelamer separated by a 1-month washout period or 3 months without sevelamer followed by 3 months with sevelamer, also separated by a 1-month washout period.

Outcomes: The primary outcome was 24-hour proteinuria (n = 49 patients). Secondary outcomes included measured GFR (using iohexol plasma clearance), office blood pressure (BP), serum lipid levels, levels of inflammation and bone metabolism biomarkers, urinary electrolyte levels, and arterial stiffness.

Results: Changes in proteinuria during the 3-month treatment with (from 1.36 [IQR, 0.77-2.51] to 1.36 [IQR, 0.77-2.60] g/24 h) or without (from 1.36 [IQR, 0.99-2.38] to 1.48 [IQR, 0.81-2.77] g/24 h) sevelamer were similar ($P = 0.1$). Sevelamer reduced urinary phosphate excretion without affecting serum phosphate levels. Sevelamer reduced C-reactive protein (CRP), glycated hemoglobin, and total and low-density lipoprotein cholesterol levels and increased high-density lipoprotein cholesterol levels without affecting levels of office BP, measured GFR, fibroblast growth factor 23, klotho, intact parathyroid hormone, serum vitamin D, or other urinary electrolytes. Results were similar in the low- and high-phosphate groups. Sevelamer was well tolerated. Adverse events were comparable between treatment periods. One case of transient hypophosphatemia was observed during treatment with sevelamer.

Limitations: Short treatment duration, lower pretreatment proteinuria than expected.

Conclusions: 3-month sevelamer treatment did not reduce proteinuria in patients with CKD on maximal RAS blockade. Amelioration of inflammation and dyslipidemia with sevelamer treatment raises the possibility that it may confer benefit in patients with CKD beyond reduction of proteinuria.

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Chronic kidney disease (CKD) has reached epidemic proportions worldwide.[1] However, the factors responsible for CKD progression are incompletely understood.[2] Proteinuria is the best predictor of glomerular filtration rate (GFR) decline in the long term,[3] and interventions aimed at achieving proteinuria remission, that is, optimal renin-angiotensin system (RAS) blockade, are able to slow progressive kidney function loss in patients with chronic proteinuric nephropathies.[4] However, in patients with residual proteinuria despite optimized RAS inhibition and achieved target blood pressure (BP), the risk for CKD progression is still substantial.[5]

Recent studies have shown that higher serum phosphate levels are associated with accelerated CKD progression. A study of 448 non–dialysis-dependent patients with CKD in the Netherlands demonstrated that higher serum phosphate levels were associated with a more rapid decline in kidney function and higher mortality.[6] Another study of 985 US veterans with CKD revealed that a 1-mg/dL increment in phosphorus level increases the risk for decline in GFR by 29%.[7] Moreover, in 1,094 African Americans, higher serum phosphate level was found to be an independent risk factor for the composite of GFR decline and end-stage kidney disease (ESKD).[8] A post hoc analysis of the Ramipril Efficacy in Nephropathy (REIN) trial found that higher baseline serum phosphate levels, even within the reference range, were associated with increased risk for progression to ESKD.[2] Importantly, this study found that higher serum phosphate levels markedly reduced the renoprotective effect of RAS blockade. Finally,

increased serum phosphate levels were found to be associated with higher degrees of proteinuria in nondiabetics with late-stage CKD.[9] Thus, elevated serum phosphate level is associated with an increased risk for CKD progression and may mitigate the beneficial effects of RAS blockade.

Despite the possible detrimental effects of increased serum phosphate levels, it is unclear whether lowering serum phosphate level reduces proteinuria and/or slows CKD progression.[10] Relevant to this, no studies have examined the effect of phosphate binders on proteinuria and/or CKD progression. Sevelamer carbonate, a calcium-free phosphate binder, is widely used to reduce serum phosphate levels in patients with CKD[11] and does so in a safe and efficacious manner.[12]

In an animal model of CKD, sevelamer was reported to diminish deterioration in kidney function and decrease proteinuria.[13] Further, Yubero-Serrano et al[14] reported that sevelamer reduces urinary albumin excretion in a subset of diabetic patients with CKD. Thus, in a prospective randomized crossover study, we investigated the antiproteinuric effects of phosphate-binding therapy with sevelamer carbonate in patients with CKD with residual proteinuria despite optimal RAS blockade.

Methods

Study Design

This was a randomized, open label, blinded–end point, crossover, phase 2 trial that recruited 53 participants from 2 Italian centers (Clinical Research Center for Rare Diseases Aldo e Cele Daccò, Ranica, Bergamo, and Bianchi-Melacrino-Morelli Hospital, Nephrology Unit, Reggio Calabria) between November 2013 and December 2014. Patients who met the selection criteria and were on maximal tolerated doses of ramipril and irbesartan were initially stratified according to serum phosphate level ≤4 or >4 mg/dL. Each group was then randomly assigned to 1 of 2 treatment sequences: (1) 3 months of treatment with sevelamer carbonate (Renvela; Sanofi-Aventis SpA), 1,600 mg, 3 times per day during meals, followed by a 1-month washout, and 3 months without sevelamer; or (2) 3 months without sevelamer, followed by a 1-month washout, and 3 months of treatment with sevelamer carbonate, 1,600 mg, 3 times daily (Fig S1).

Randomization was centralized at the Laboratory of Biostatistics of the Clinical Research Center for Rare Diseases Aldo e Cele Daccò Villa Camozzi, Ranica, Bergamo, of the Mario Negri Institute for Pharmacological Research IRCCS under the responsibility of an independent investigator. The study protocol was approved by the ethics committees of both centers, and a written informed consent was obtained from each patient enrolled in the study.

Participants

Eligible individuals were adults older than 18 years with estimated GFRs > 15 mL/min/1.73 m^2 (as calculated using the 4-variable Modification of Diet in Renal Disease [MDRD] Study equation) and urinary protein excretion ≥ 0.5 g/24 h despite optimized therapy with RAS inhibitors who were not receiving concomitant therapy with phosphate binders. Main exclusion criteria were serum phosphate level outside the range of 2.5 to 5.5 mg/dL, serum calcium level < 7.5 or >10.5 mg/dL, and serum parathyroid hormone (PTH) level > 250 pg/mL in patients who were not receiving therapy with vitamin D (calcitriol or paricalcitol) or calcimimetics for at least 3 months at the time of enrollment. Reasons for exclusion before randomization and during the study follow-up are reported in Item S1.

Potentially eligible patients not receiving maximum tolerated doses of dual-therapy RAS blockade, specifically with ramipril and irbesartan, entered a 2 month run-in treatment period: angiotensin-converting enzyme (ACE) inhibition with ramipril was progressively uptitrated (2.5 to 10 mg/d) for 1 month, followed by an additional month of angiotensin receptor blockade with irbesartan (75-300 mg/d) on top of the maximum tolerated ACE inhibition achieved. The maximal tolerated dose was defined as the dose that reduced BP to <120/80 mm Hg and proteinuria to protein excretion < 0.3 g/24 h in the absence of symptomatic hypotension, hyperkalemia (serum potassium persistently >5.5 mEq/L), metabolic acidosis and hyperglycemia in patients with diabetes, or serum creatinine level increase >30% versus baseline. Patients were included who did not tolerate maximal labeled doses of a single agent or both drugs in combination. No adjustments in ACE inhibitor/angiotensin receptor blocker dosing were made during the study unless deemed clinically appropriate.

Before enrollment, patients were recommended to have protein intake of ~0.8 mg per kilogram of body weight and sodium intake < 100 mEq/d. A dietician monitored salt and protein intake at inclusion and throughout the entire study period, and no systematic changes in protein and salt intake were introduced thereafter.

Outcome Variables

Efficacy Parameters

The protocol-specified primary efficacy variable was 24-hour proteinuria. Primary outcome analysis evaluated changes in 24-hour proteinuria at the end of the 2 treatment periods with sevelamer or without sevelamer compared to each pretreatment period. Three consecutive 24-hour proteinuria measurements were obtained at each visit and the mean of the 3 samples was used.

Secondary efficacy measures included differences between pretreatment and end-of-treatment measured GFRs as assessed using iohexol plasma clearance[15]; office systolic and diastolic BP, 24-hour urine calcium, phosphate, magnesium, sodium, urea, and albumin; biomarkers of mineral metabolism, including serum levels of 25-hydroxyvitamin D_3, 1,25-dihydroxyvitamin D_3, calcium,

phosphorus, intact PTH, alkaline phosphatase, intact fibroblast growth factor 23 (FGF-23), and klotho; serum levels of markers of inflammation (high-sensitivity C-reactive protein [CRP] and interleukin 6); serum lipids (total, high- [HDL] and low-density lipoprotein [LDL] cholesterol and triglycerides); and parameters of arterial stiffness (pulse wave velocity and augmentation index as assessed noninvasively using applanation tonometry).

All blood and urine tests were done at each nephrology unit except for serum klotho, 1,25-dihydroxyvitamin D_3, and FGF-23, which were centralized at the Department of Cardiovascular, Respiratory, Nephrologic, Anesthetic and Geriatric Sciences of the Sapienza University of Rome, and iohexol plasma clearance, at the Mario Negri Research Institute. Klotho was measured using a commercial enzyme-linked immunosorbent assay kit (Immuno Biological Laboratories Co, Ltd),[16] 1,25-dihydroxyvitamin D_3 was assayed using a fully automated extraction-free chemiluminescent immunoassay commercial kit (DiaSorinInc),[17] and FGF-23 was assayed using a fully automated chemiluminescent immunoassay detecting the whole intact and biologically active molecule (DiaSorinInc).[18]

Safety Parameters

Safety parameters were evaluated at baseline and at monthly intervals throughout the study period and included vital signs, complete blood cell count, serum electrolytes (potassium, sodium, magnesium, phosphate, and calcium), serum chemistry (glucose, urea, creatinine, and liver transaminases), and adverse events. Serum phosphate and calcium levels were monitored weekly during the first month of each treatment period and within 1 week after any adjustment to sevelamer carbonate or dual RAS blockade dose(s). The dose of sevelamer carbonate was adjusted as deemed appropriate by the investigator in charge of the patient to maintain phosphate levels close to but never <2.5 mg/dL. If serum phosphate level was <2.5 mg/dL, the sevelamer dose was decreased to 800 mg and phosphate levels were monitored within 1 week. To avoid the risk for vitamin D depletion during sevelamer treatment, all participants were supplemented with calcidiol, 400 IU/d (Didrogyl [Bruno Farmaceutici S.p.A.]; 2 drops per day), at randomization.

Statistical Analysis

Baseline characteristics (Table 1) were compared according to Wilcoxon rank sum test, χ^2, or Fisher test as appropriate to the random allocation crossover sequence (ie, sequence A: sevelamer–without sevelamer, sequence B: without sevelamer–sevelamer) and according to serum phosphate strata (ie, levels >4 or ≤4 mg/dL).

Table 1. Baseline Characteristics of All Randomly Assigned Patients and According to Treatment Allocation Sequence

Characteristics	Overall (N = 53)	Sevelamer–Without Sevelamer (n = 26)	Without Sevelamer–Sevelamer (n = 27)	*P*
Age, y	55 ± 17	54 ± 17	56 ± 17	0.6
Male sex	42 (79%)	21 (81%)	21 (78%)	0.8
White	52 (98%)	26 (100%)	26 (96%)	0.3
Diabetic	14 (26%)	8 (31%)	6 (22%)	0.6
Body mass index, kg/m^2	27 ± 4	27 ± 4	27 ± 4	0.7
Systolic BP, mm Hg	128 ± 22	126 ± 23	129 ± 21	0.6
Diastolic BP, mm Hg	73 ± 10	72 ± 7	73 ± 12	0.6
Pulse rate, beats/min	69 ± 11	71 ± 10	67 ± 12	0.3
Creatinine, mg/dL	1.60 [1.00-2.30]	1.35 [0.97-2.21]	1.76 [1.38-2.35]	0.1
Phosphate, mg/dL	3.8 ± 0.6	3.8 ± 0.7	3.8 ± 0.6	0.7
Calcium, mg/dL	9.2 ± 0.4	9.2 ± 0.4	9.1 ± 0.4	0.3
PTH, pg/dL	70 ± 33	68 ± 39	72 ± 28	0.7
Magnesium, mg/dL	1.97 ± 0.22	1.97 ± 0.24	1.96 ± 0.20	0.9
Cholesterol, mg/dL	181 ± 38	178 ± 30	183 ± 44	0.6
LDL cholesterol, mg/dL	113 ± 33	111 ± 23	114 ± 41	0.8
Triglycerides, mg/dL	114 [88-159]	126 [88-166]	109 [86-147]	0.5
Albumin, g/dL	3.6 ± 0.4	3.7 ± 0.3	3.5 ± 0.5	0.05
Hemoglobin, g/dL	12.9 ± 1.8	13.2 ± 1.8	12.7 ± 1.8	0.4
Urine protein, g/24 h	1.54 [0.97-2.59]	1.28 [0.91-2.31]	2.25 [1.03-2.84]	0.2
Urine albumin, μg/min	829 [481-1,372]	655 [379-1,229]	1,087 [552-1,583]	0.1
mGFR, mL/min/1.73 m^2	49.3 ± 23.5	55.5 ± 23.7	43.3 ± 22.1	0.06
Fractional albumin clearance, ×10^{-5}	43 [18-93]	25 [12-75]	52 [29-101]	0.1

Note: Data are mean ± standard deviation, median [interquartile range], or number (percentage). Chemistries are serum values unless stated otherwise. Conversion factors for units: creatinine in mg/dL to μmol/L, ×88.4; phosphate in mg/dL to mmol/mol, ×0.3229; calcium in mg/dL to mmol/L, ×0.2495; magnesium in mEq/L to mmol/L, ×0.5; cholesterol (total and LDL) in mg/dL to mmol/L, ×0.02586; triglycerides in mg/dL to mmol/L, ×0.01129. *P* values are obtained using χ^2, *t*, or Wilcoxon signed rank tests. Abbreviations: BP, blood pressure; LDL, low-density lipoprotein; mGFR, measured glomerular filtration rate; PTH, parathyroid hormone.

Within-patient changes in the primary efficacy variable, 24-hour urinary protein excretion, were evaluated between post- versus pre-sevelamer and post– versus pre–without sevelamer period and between treatments. Treatment period changes were compared using Wilcoxon signed rank test within the study group considered as a whole (as shown in Table 2) and within 2 high- and low-phosphate strata (as shown in Fig 2), assuming no period effect and no carryover effect (ie, $P > 0.05$ for both). All remaining secondary within-group comparisons (in the entire study group and in each phosphate stratum considered separately) were carried out using paired t test, Wilcoxon signed rank test, or McNemar test.

Carryover effect and period effect were tested by means of a mixed-effect model for repeated measures applied to log-transformed proteinuria (PROC MIXED; SAS, version 9.2; SAS Institute Inc) with sequence, period (ie, visit 3 = month 0, visit 4 = 3 months, visit 5 = 4 months, and visit 6 = 7 months), and treatment (ie, treatment 1 = sevelamer, treatment 2 = without sevelamer) as fixed effects and participant nested in sequence as random effect.[19]

On the basis of the REIN data in patients with proteinuric CKD and residual proteinuria with protein excretion > 0.5 g/24 h after the 3-month ramipril therapy, urinary protein excretion at baseline was expected to average 2.8 ± 2.6 g/24 h. Assuming a reduction in urinary protein excretion averaging 1.0 ± 2.1 g/24 h (standard deviation based on REIN cohort), the number of patients required to detect a statistically significant difference (alpha = 0.05) in urinary protein excretion between sevelamer treatment and without sevelamer ranged from 37 (power, 80%) to 49 (power, 90%), or 41 to 54, respectively, assuming 10% dropout. Thus, a sample size of 50 patients was estimated sufficient to detect the hypothesized treatment effect.

All analyses were done according to the intention-to-treat principle, that is, considering all participants randomly assigned to sevelamer–without sevelamer sequence or without sevelamer–sevelamer sequence who took at least 1 dose of study drug and who had an efficacy measurement after the first study drug, without imputation of missing data. Results were expressed as mean ± standard deviation, median with interquartile range, or number and percent. For multiple comparisons of proteinuria between the high– or low–serum phosphate groups, significance level was set at a 0.025 (Bonferroni correction). Normality assumption was assessed by means of Shapiro-Wilk test. SAS, version 9.2, and Stata (StataCorp), version 12, were used for all analyses. All *P* values were 2 sided.

Results

Design and Participant Characteristics

Seventy-two patients were enrolled in the study (55 from Bergamo and 17 from Reggio Calabria). Detailed information about excluded, classified, randomly assigned, and analyzed patients is provided in Figure 1. Final safety analyses included 53 patients and per-protocol efficacy analyses included 49 patients.

At randomization, 41 patients were receiving dual RAS blockade with ramipril and irbesartan but 12 remained on single RAS inhibition with ramipril (n = 7, except 1 taking benazepril) or irbesartan (n = 5, except 1 taking valsartan; Table S1). Adherence to study drug treatment was high, as assessed using pill counts at every visit.

Overall, 25% had diabetes. Baseline characteristics of patients randomly assigned to the treatment sequences were similar (Table 1) with the exception of serum albumin and mGFR values, which tended to be lower in the without sevelamer–sevelamer treatment sequence.

Patients' characteristics (including mGFR) according to phosphate strata at randomization were similar (Table S2) with the only exception of serum creatinine levels. Though urinary protein and albumin excretion were higher in the high–serum phosphate stratum, the difference between strata did not achieve statistical significance.

Baseline proteinuria was similar among participants who performed a 2-month, 1-month, or no run-in period (Table S3). Additional analysis showed that urinary proteinuria at eligibility evaluation did not significantly differ from baseline values, regardless of the duration of the run-in period (Table S4).

Primary Outcome

In the intention-to-treat analysis, no difference was observed in change in 24-hour proteinuria between the 2 periods with sevelamer or without sevelamer (Fig 2). Similar findings were observed within the high– or low–serum phosphate strata considered separately (Fig 2) and when analyses were restricted to the first treatment period ($P = 0.3$). No period effect ($P = 0.3$) or carryover effect ($P = 0.7$) was observed. Similarly, no difference was observed when 4 patients were excluded from the analysis, 1 due to a serious adverse event (colon cancer) and 3 due to nonserious adverse events (relapse of nephrotic syndrome in 2 patients and symptomatic hypothyroidism in 1 patient).

Secondary Outcomes

Cardiovascular Parameters

Sevelamer treatment did not affect office systolic BP, while office diastolic BP increased with sevelamer treatment. However, this change did not significantly differ in the sevelamer versus without sevelamer comparison (Table 2).

Mineral Metabolism

Twenty-four–hour urinary phosphate excretion decreased during sevelamer treatment but not during the without sevelamer period (Fig 3, right panels), and changes in urinary phosphate excretion were

Table 2. Changes in Primary and Secondary End Points Within and Between Sevelamer and Without Sevelamer Periods

Characteristics	Sevelamer		P	Without Sevelamer		P	Sevelamer vs Without Sevelamer[c]	P
	Pre	Post		Pre	Post			
Primary end point								
Urinary protein, g/24 h	1.36 [0.77 to 2.51]	1.36 [0.77 to 2.60]	0.1	1.36 [0.99 to 2.38]	1.48 [0.81 to 2.77]	0.5	0.31 [−0.43 to 0.81]	0.1
Secondary end points								
Systolic BP, mm Hg[b]	124 ± 20	125 ± 18	0.4	127 ± 19	124 ± 12	0.2	0.03 ± 0.14	0.1
Diastolic BP, mm Hg[b]	72 ± 9	74 ± 9	0.01	73 ± 10	73 ± 9	0.9	2.12 ± 10.33	0.2
Arterial stiffness parameters								
PWV, m/s	8.70 ± 2.96	8.83 ± 3.06	0.5	9.35 ± 3.45	8.88 ± 2.85	0.1	0.20 ± 2.97	0.6
AIx	14.43 [7.31 to 22]	14.12 [6.68 to 21.07]	0.5	15.43 [4.60 to 23.00]	15.07 [4.60 to 23.85]	0.7	5.14 [−10.61 to 13.00]	0.3
Mineral metabolism markers								
Calcium, mg/dL	9.1 ± 0.4	9.1 ± 0.4	0.4	9.1 ± 0.4	9.1± 0.4	0.5	−0.03 ± 0.56	0.7
Phosphate, mg/dL	3.9 ± 0.7	3.9 ± 0.6	0.7	3.8 ± 0.6	3.9 ± 0.7	0.3	−0.04 ± 0.81	0.7
Magnesium, mg/dL	2.0 ± 0.2	2.0 ± 0.2	0.3	2.0 ± 0.2	2.0 ± 0.2	0.4	0.02 ± 0.21	0.6
PTH, pg/dL	70.6 ± 27.7	74.5 ± 36.6	0.3	70.5 ± 38.1	75.2 ± 45.9	0.2	−1.4 ± 42.5	0.6
25(OH)D, ng/mL	23.3 ± 16.1	27.0 ± 16.4	0.03	24.7 ± 17.2	27.5 ± 16.9	0.08	1.1 ± 17.9	0.6
$1,25(OH)_2D$, pg/mL	30.7 ± 12.9	30.0 ± 11.8	0.6	32.1 ± 12.8	35.2 ± 15.7	0.03	−4.0 ± 12.9	0.06
Acid-base balance								
Base excess[a]	−0.6 [−3.8 to 1.9]	−1.1 [−2.6 to 1.6]	0.3	0.2 [−2.0 to 2.2]	−0.6 [−3.0 to 1.4]	0.04	0.95 [−0.4 to 2.8]	0.02
pH[a]	7.32 ± 0.04	7.34 ± 0.04	0.01	7.33 ± 0.06	7.34 ± 0.04	0.2	0.01 ± 0.07	0.1
Glucose, protein, & lipid profile								
Glucose, mg/dL	104 ± 35	102 ± 44	0.6	103 ± 39	102 ± 36	0.8	−2.6 ± 56	0.8
HbA_{1c}, mmol/mol[a,d]	5.35 [4.56 to 7.06]	4.88 [4.42 to 7.19]	0.002	5.19 [4.74 to 6.45]	5.86 [5.04 to 7.31]	0.06	−0.57 [−1.08 to −0.18]	0.001
Albumin, g/dL	3.6 ± 0.4	3.5 ± 0.4	0.03	3.5 ± 0.5	3.5± 0.4	0.3	−0.08 ± 0.39	0.2
Proteins, g/dL	6.6 ± 0.5	6.5 ± 0.5	0.02	6.5 ± 0.6	6.5 ± 0.5	0.08	−0.05 ± 0.54	0.5
UN, mg/dL	77 ± 34	76 ± 33	0.8	74 ± 34	82 ± 41	0.01	−0.04 ± 0.81	0.01
Cholesterol								
Total, mg/dL	180 ± 39	139 ± 29	<0.001	177 ± 39	173 ± 39	0.3	−33.5 ± 43.3	<0.001
HDL, mg/dL	43 ± 11	45 ± 11	0.06	45 ± 12	44 ± 13	0.5	2.9 ± 9.1	0.04
LDL, mg/dL	109 ± 30	73 ± 23	<0.001	108 ± 36	104 ± 32	0.2	−30.5 ± 33.8	<0.001
TG, mg/dL	150 ± 97	141 ± 88	0.6	134 ± 81	143 ± 88	0.3	−13.1 ± 67.6	0.3
Inflammatory markers								
CRP, mg/L	1.86 [0.74 to 3.47]	1.02 [0.55 to 1.94]	0.001	1.75 [0.61 to 4.36]	1.53 [0.76 to 2.93]	0.9	−0.43 [−2.32 to 0.74]	0.02
IL-6, pg/mL	4.29 ± 5.68	4.41 ± 7.30	0.9	4.29 ± 5.33	4.98 ± 8.65	0.9	−0.40 ± 10.8	0.9

(Continued)

Table 2 (Cont'd). Changes in Primary and Secondary End Points Within and Between Sevelamer and Without Sevelamer Periods

Characteristics	Sevelamer		P	Without Sevelamer		P	Sevelamer vs Without Sevelamer[c]	P
	Pre	Post		Pre	Post			
24-h urinary parameters								
Sodium, mEq/24 h	153 ± 55	144 ± 59	0.3	143 ± 56	142 ± 55	0.8	−7.1 ± 78.4	0.5
Calcium, mg/24 h	38.9 [27.3 to 69.3]	36.4 [27.8 to 61.8]	0.7	39.1 [28.8 to 61.6]	40.3 [27.7 to 66.7]	0.9	−0.5 [−12.3 to 12.7]	0.9
Magnesium, mg/24 h	85 ± 39	91 ± 44	0.08	88 ± 36	84 ± 37	0.4	11.0 ± 32.8	0.04
Phosphate, mg/24 h	743 ± 252	632 ± 180	<0.001	772 ± 246	773 ± 245	0.9	−94 ± 223	0.005
Urea, mg/24 h	20.6 [13.6 to 25.2]	22.5 [16.9 to 25.5]	0.2	21.1 [15.4 to 25.0]	21.0 [18.4 to 24.5]	0.9	1.13 [−2.2 to 4.3]	0.2
Albumin, µg/24 h	666 [355 to 1,463]	788 [425 to 1,347]	0.2	667 [454 to 1,197]	669 [471 to 1,354]	0.7	129 [−217 to 366]	0.2
mGFR, mL/min/1.73 m^2[b]	48 ± 22	47 ± 22	0.2	47 ± 21	47 ± 22	0.3	−0.03 ± 0.22	0.8

Note: All data expressed as mean ± standard deviation or median [interquartile range]. Conversion factors for units: calcium in mg/dL to mmol/L, ×0.2495; phosphate in mg/dL to mmol/L, ×0.3229; 25(OH)D in ng/mL to nmol/L, ×2.496; 1,25(OH)$_2$D in pg/mL to pmol/L, ×2.6; cholesterol (total, HDL, and LDL) in mg/dL to mmol/L, ×0.02586; TG in mg/dL to mmol/L, ×0.01129. *P* values are from paired *t* or Wilcoxon signed rank tests.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; Alx, augmentation index; BP, blood pressure; CRP, C-reactive protein; HbA$_{1c}$, glycated hemoglobin; HDL, high-density lipoprotein; IL-6, interleukin 6; LDL, low-density lipoprotein; mGFR, measured glomerular filtration rate; PTH, parathyroid hormone; PWV, pulse wave velocity; TG, triglycerides; UN, urea nitrogen.

Chemistries are serum values unless stated otherwise:

[a]whole-blood sample.

Intention-to-treat analysis except:

[b]per protocol;

[c]change between pre and post in the sevelamer period (n = 53) versus change between pre and post in the without sevelamer period (n = 49);

[d]diabetic patients only (n = 14).

significantly different between treatment periods. However, serum phosphate levels did not change during both treatment periods (Fig 3, left panels). Sevelamer treatment did not change FGF-23 levels in both strata (Fig 4, left panels). Serum klotho levels increased during the without sevelamer period regardless of initial serum phosphate levels; however, this change was not significantly different between treatment periods (Fig 4, right panels).

Other data for markers of mineral metabolism are shown in Table 2.

Other Outcomes

Base excess decreased during the period without sevelamer and this change significantly differed between treatment groups. However, change in venous pH, which increased after sevelamer, was not significantly different between the 2 treatment periods (Table 2).

Sevelamer significantly reduced glycated hemoglobin (HbA_{1c}), total and LDL cholesterol, and CRP levels. Serum urea nitrogen levels significantly increased during the period without sevelamer and did not change during the sevelamer treatment periods; this change was significantly different between the 2 treatment periods (Table 2).

While mGFR and 24-hour excretion and fractional clearance of urinary metabolites did not change after sevelamer treatment, 24-hour magnesium excretion tended to increase. This change was associated with an increase in fractional excretion and was significantly different between treatment periods.

Dietary Assessment

Dietary protein and sodium intakes assessed using dietary diary recording did not change during both treatment periods.

Multiple Regression Analysis of the Primary Outcome

The effect of baseline parameters on sevelamer-associated changes in 24-hour proteinuria was assessed using multivariable analysis, adjusting for sex, age, mGFR, and serum phosphate, PTH, FGF-23, and klotho levels (n = 46). Only klotho level showed a significant inverse correlation. However, the magnitude of the association was very small (−0.00393; P = 0.03).

Safety

There were no major safety signals except for a non–clinically relevant increase in alkaline phosphatase levels during the sevelamer versus without sevelamer treatment (64 ± 18 vs 68 ± 20 IU/L pre- vs post-sevelamer; P < 0.02). Most adverse events were nonserious (Table S5). A colon cancer (Table 3) and 3 nonserious adverse events resulted in patient withdrawal; all were deemed unlikely to be related to sevelamer treatment. There were 3 additional serious adverse

events likely unrelated to sevelamer (Table 3). Nine events in 7 patients were possibly treatment-related adverse events. Of these, 1 was constipation and 1 was hypophosphatemia that recovered after sevelamer downtitration (Table S5).

Discussion

The current study shows that sevelamer carbonate treatment reduced urinary phosphate excretion but did not alter serum phosphate concentrations in patients with proteinuric CKD. It seems likely that reduced gut phosphate absorption was compensated for by increased kidney phosphate retention, thereby maintaining serum phosphate levels. These findings agree with those in patients with moderate or advanced CKD wherein sevelamer lowered urinary phosphate excretion with no or only a modest reduction in serum phosphate levels.[20,21] Finally, it should be noted that a single serum phosphate value may not accurately reflect time-averaged dietary phosphate absorption.[22]

Despite lowering phosphaturia, sevelamer did not reduce proteinuria over a 3-month period in patients with CKD and residual proteinuria who were receiving maximal tolerated doses of RAS inhibitors. The lack of a proteinuria-lowering effect was observed in the total cohort and in the high– and low–serum phosphate strata. Similarly, albuminuria and protein and albumin fractional clearances were unaffected by sevelamer treatment. The reasons why sevelamer failed to reduce proteinuria are speculative. Of note, antiproteinuric agents such as ACE inhibitors and angiotensin receptor blockers consistently reduce intraglomerular pressure[23]; however, sevelamer has not been shown to affect glomerular hemodynamics or BP (see discussion below).

Sevelamer's failure to reduce proteinuria does not preclude a renoprotective effect for this agent. Recent studies have shown that sevelamer reduces inflammatory marker levels, improves endothelial function, and reduces uremic toxins in patients with CKD.[24,25] In addition, sevelamer reduced HbA_{1c} and advanced glycation end product levels in patients with diabetic kidney disease[14,26] and delayed dialysis inception in the INDEPENDENT-CKD study through mechanisms hypothesized to involve CRP and total and LDL cholesterol level reductions.[27] We found that sevelamer significantly reduced HbA_{1c}, CRP, and cholesterol (total and LDL) levels and increased HDL cholesterol levels, consistent with previous findings.[28-31] Thus, although sevelamer did not reduce proteinuria in the current study, these observations support the hypothesis that sevelamer may exert renoprotection through mechanisms that are independent of proteinuria reduction.

Similarly to previous reports,[21,32] we did not observe an effect of sevelamer on serum calcium level, urinary calcium excretion, 25-hydroxyvitamin D_3 level, and 1,25-dihydroxyvitamin D_3 level. In contrast, we observed no effect on PTH levels, unlike others.[21] Unlike the situation for calcium metabolism, we found that sevelamer increased urinary magnesium excretion and tended to increase magnesium fractional clearance without changes in magnesium levels. In normal rats, but not healthy humans, sevelamer has been reported to increase urinary calcium and magnesium excretion by modulating PTH levels without changing serum calcium and magnesium

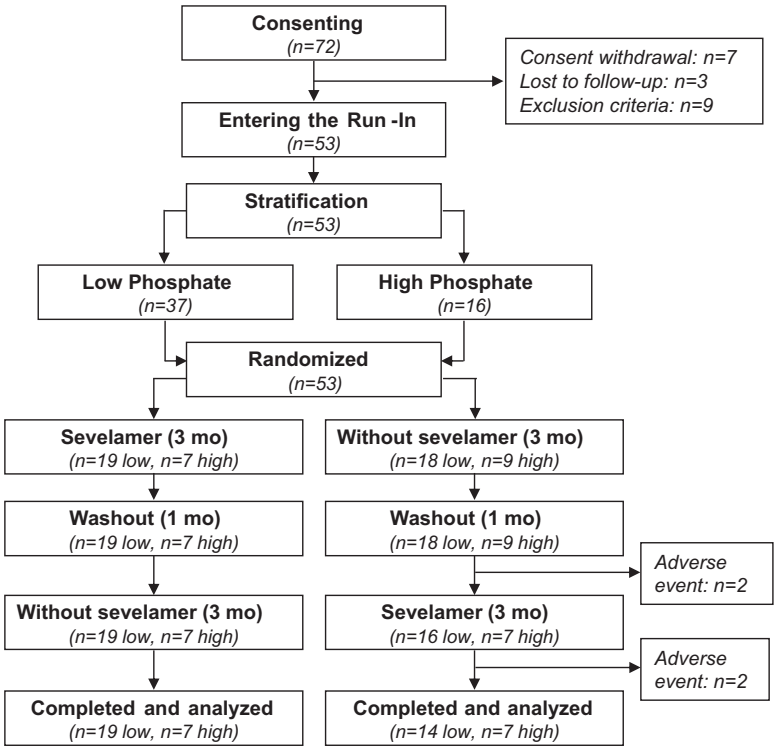

Figure 1. Study design flowchart.

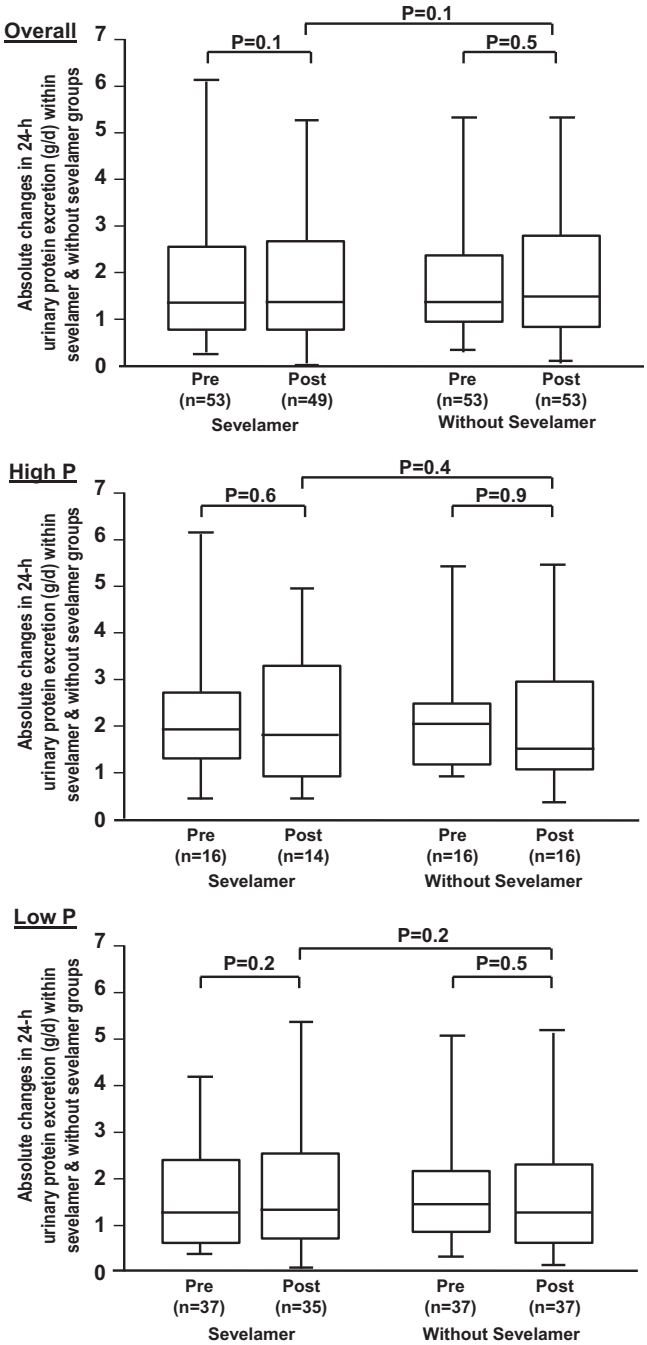

Figure 2. Primary efficacy outcome: Change in 24-hour urine protein excretion (by intention-to-treat analysis) in: (A) entire cohort, (B) high–serum phosphate (P) group, and (C) low–serum P group.

levels.[33,34] It is unlikely that changes in vitamin D levels were involved because calcidiol supplements were given to all patients. Speculatively, sevelamer may have increased gut magnesium absorption due to binding free anions.

Sevelamer did not alter serum klotho and intact FGF-23 levels, while multivariate analysis revealed no association of baseline FGF-23 levels and minimal association of baseline klotho levels with sevelamer-associated changes in proteinuria. Reported sevelamer effects on these biomarkers are conflicting. In hemodialysis patients or patients with advanced CKD, sevelamer has been reported to reduce serum FGF-23 levels when compared with other phosphate binders.[25] However, a recent double-blind placebo-controlled study with 3-month sevelamer treatment in 78 patients with stages 3 to 4 CKD failed to find a significant treatment effect on the primary outcomes of serum FGF-23 and klotho levels[35] (unfortunately, data for proteinuria or albuminuria were not reported).

Despite unaltered mGFRs, serum urea nitrogen levels increased in the group without sevelamer, but not the sevelamer treatment group. The tendency to increase dietary protein intake during the treatment periods may explain the elevated urea levels in the period without sevelamer. However, why an increase in urea levels was not observed in the sevelamer treatment period is unknown. It is conceivable that sevelamer affects gut protein or amino acid absorption as well as urea metabolism.

Base excess decreased significantly in the without sevelamer as compared to the sevelamer period, particularly in patients in the high phosphate stratum. Notably, sevelamer has been reported to increase serum bicarbonate levels.[11] Unfortunately, this effect did not translate into significant changes in venous pH. However, whether this effect may be linked to the reduced absorption of anions or other molecules responsible for uremic metabolic acidosis and whether it may be of greater magnitude with longer treatment duration should be investigated.

Sevelamer did not change mGFR or office BP. These findings are generally consistent with previous reports,[36] although Chue et al[32] described a reduction in systolic BP after 4 weeks of sevelamer treatment. Sevelamer did not change arterial stiffness parameters. In contrast, Takenaka and Suzuki[37] reported that sevelamer prevented an increase in pulse wave velocity in 15 hemodialysis patients. However, our patients had almost normal pulse wave velocities at baseline.

Interpretation of the current study should take several issues into consideration.

1. The study could be underpowered to detect an effect of sevelamer on proteinuria. Sample size was estimated on the basis of the REIN trial, which involved a similar patient population and duration of treatment. However, patients in the current study had lower baseline proteinuria (protein excretion, ~1.9 g/d) compared to REIN (~2.8 g/d), likely due to optimal (double) RAS blockade. Nonetheless, in the absence of even a trend toward sevelamer-associated proteinuria reduction, it seems unlikely that a real effect was missed, at least in patients with mild residual proteinuria (Tables S3 and S4).
2. Limited treatment duration may have reduced the opportunity to find effects of sevelamer therapy.
3. Hyperphosphatemic patients may have a different proteinuric response to sevelamer compared with patients with normal serum phosphate levels. Despite stratifying

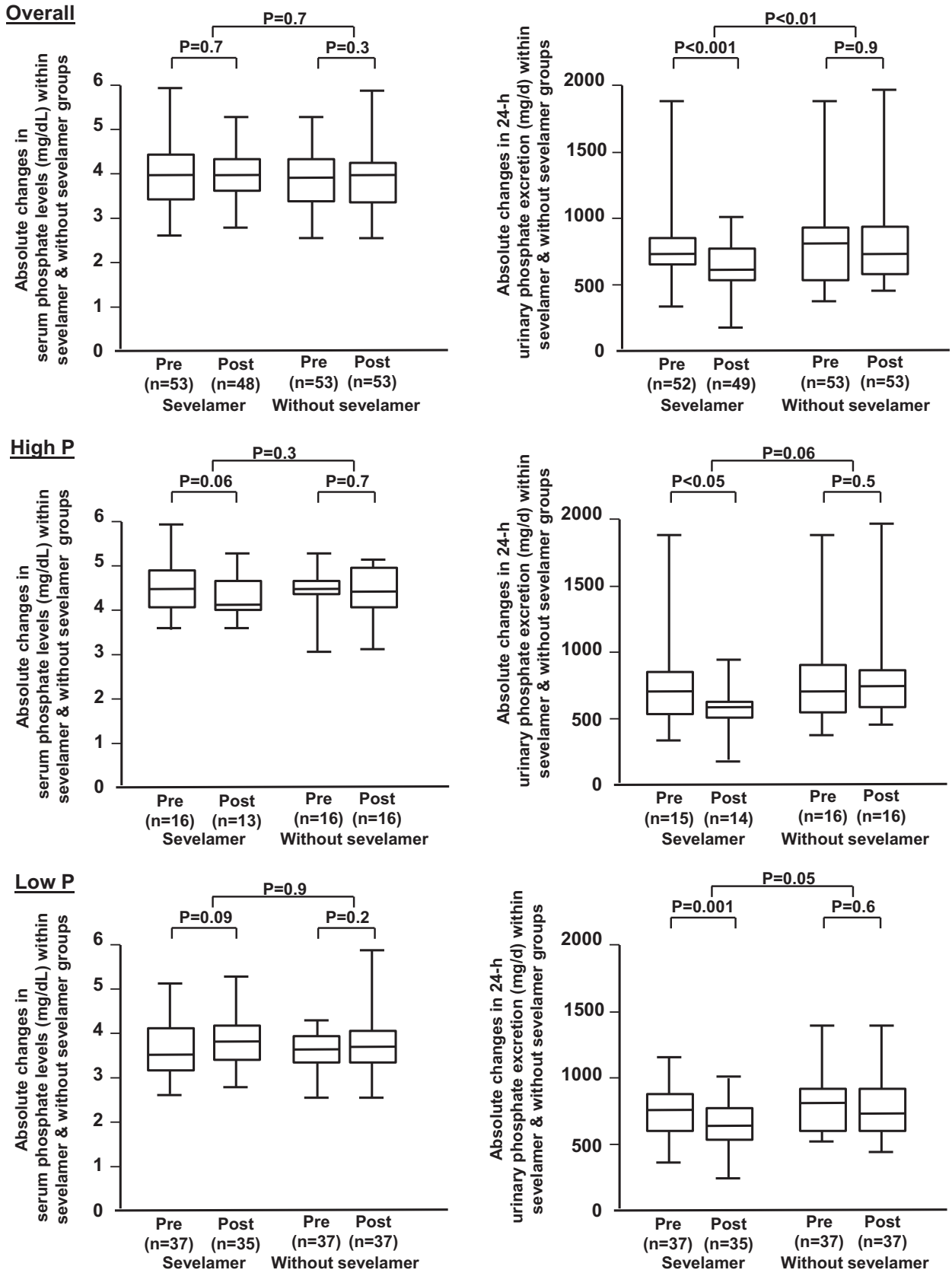

Figure 3. Changes in (left panels) serum phosphate levels and (right panels) urine phosphate excretion in: (A) entire cohort; (B) high–serum phosphate (P) group; and (C) low–serum P group.

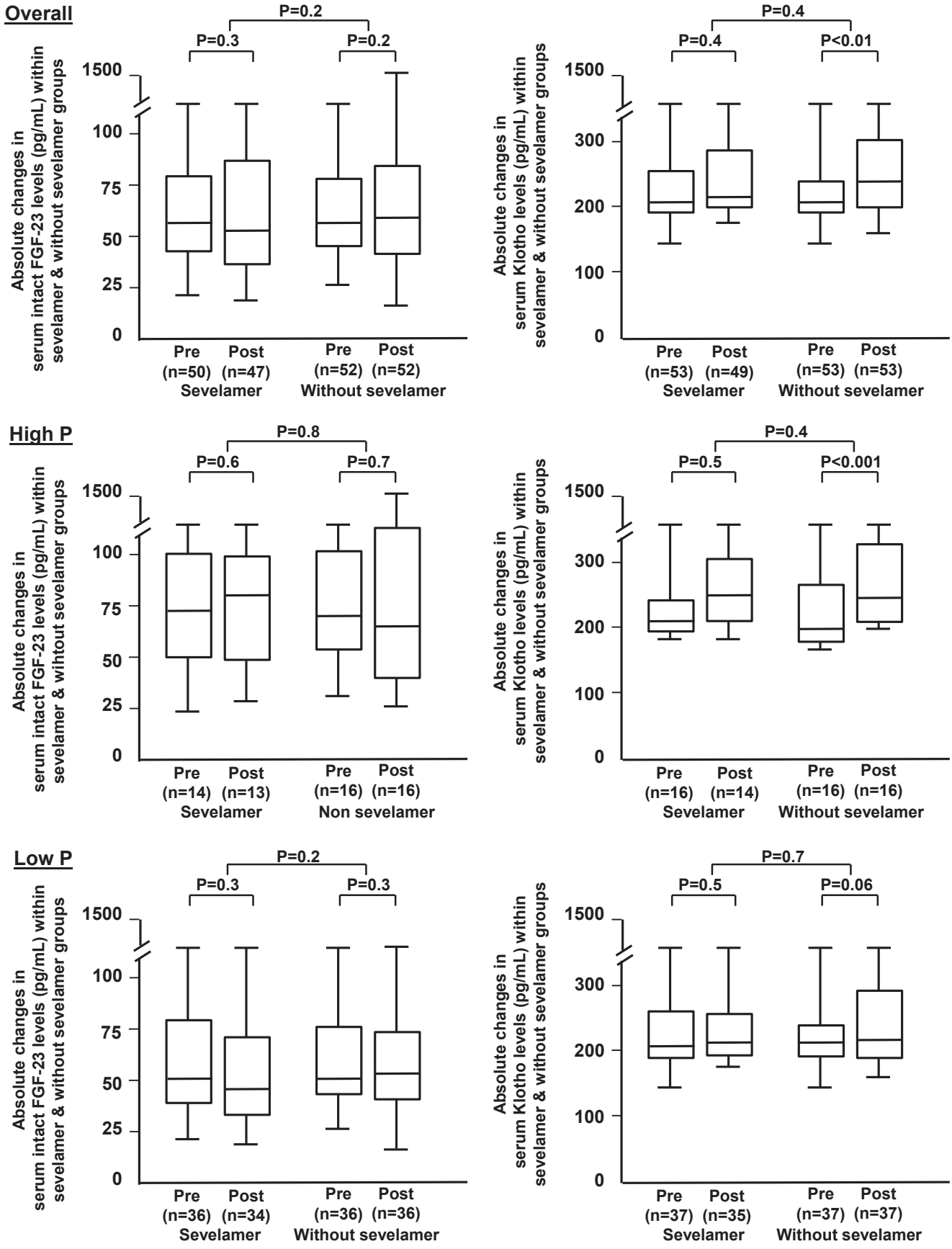

Figure 4. Changes in (left panels) fibroblast growth factor 23 (FGF-23) and (right panels) serum klotho levels in: (A) entire cohort; (B) high–serum phosphate group; and (C) low–serum phosphate group.

Table 3. Serious Adverse Events in the Overall Study and According to Treatment Period

	Overall (N = 53)	Sevelamer (n = 53)	Without Sevelamer (n = 53)
Total	4 (8%)	2 (4%)	2 (4%)
By type			
Hemorrhagic stroke	1 (2%)	1 (2%)	0 (0%)
Ankle fracture	1 (2%)	1 (2%)	0 (0%)
Colon adenoma	1 (2%)	0 (0%)	1 (2%)
Anal abscess	1 (2%)	0 (0%)	1 (2%)

Note: Colon adenoma, anal abscess, and ankle fracture are grade 3 and hemorrhagic stroke is grade 4; anal abscess and ankle fracture occurred before initiation of study drug. No patient experienced more than one serious adverse event. McNemar test performed.

patients according to baseline serum phosphate levels before randomization, a minority were in the high–phosphate level stratum. Furthermore, our stringent selection criteria did not allow the inclusion of patients with serum phosphate levels > 5.5 mg/dL. Thus, power to detect any treatment effect in the patient population with abnormal or even high-normal phosphate levels may have been reduced. This may limit the generalizability of the study findings to the global CKD patient population.

4. Vitamin D supplementation at study commencement might have altered mineral metabolism and bone turnover and as a consequence, interfered with study results.
5. Almost all patients were of European ancestry in the current study. A previous study found that sevelamer-induced albuminuria reduction in diabetic patients with CKD was only evident in individuals of non-European ancestry.[14] Although only 25% of patients had diabetes in our study, the possibility must be considered that this race-specific effect is generalizable to patients with other forms of CKD.
6. Although the current study design aimed at removing confounding effects through optimal BP control, maximum tolerable RAS inhibition, and strictly monitored protein and salt dietary intake, other variables may have affected results.

In summary, the current study found that 3 months of treatment with sevelamer did not reduce proteinuria in patients with CKD on optimal dual RAS blockade. In contrast, sevelamer reduced serum urea, HbA_{1c}, CRP, and LDL and total cholesterol levels and increased HDL cholesterol levels. Thus, additional studies are necessary to assess sevelamer effect on proteinuria and determine whether longer term treatment with sevelamer slows CKD progression and reduces the increased cardiovascular risk of patients with CKD, potentially through its potential anti-inflammatory effects.

Supplementary Material

Supplementary File (PDF)

Figure S1: Study design.

Item S1: Detailed exclusion criteria and reasons for withdrawal.

Table S1: Concomitant medications at baseline according to treatment allocation.

Table S2: Baseline characteristics of all randomized patients and according to the serum phosphate stratum.

Table S3: Baseline proteinuria according to run-in period length.

Table S4: Urinary proteins at eligibility and at baseline according to run-in period length.

Table S5: Nonserious adverse events in the overall study group and according to treatment period.

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Data Sharing: Upon request the authors will provide access to individual participant data, which will be made available to study participants, further patients not enrolled in the present study, patients' associations, and researchers whose proposals meet methodologically sound research criteria to be discussed and shared with the study authors. Data may be requested up to 24 months after study publication. Requests for access to the study data can be submitted via e-mail to Dr Perna (annalisa.perna@marionegri.it), head of the Laboratory of Biostatistics of the Department of Renal Medicine of the Istituto di Ricerche Farmacologiche Mario Negri IRCCS.

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