Phosphate May Promote CKD Progression and Attenuate Renoprotective Effect of ACE Inhibition

Carmine Zoccali,*† Piero Ruggenenti,‡ Annalisa Perna,‡ Daniela Leonardis,† Rocco Tripepi,† Giovanni Tripepi,† Francesca Mallamaci,*† and Giuseppe Remuzzi,‡ for the REIN Study Group

*Nephrology, Dialysis and Transplantation Unit, Ospedali Riuniti, and †CNR-IBIM, Reggio Calabria, Italy; ‡Unit of Nephrology, Azienda Ospedaliera "Ospedali Riuniti di Bergamo," and Mario Negri Institute for Pharmacological Research, Bergamo, Italy

ABSTRACT

Phosphate may promote the onset and progression of chronic nephropathies. Here we evaluated the relationships between baseline serum phosphate levels, disease progression, and response to ACE inhibition in 331 patients with proteinuric nephropathies in the prospective Ramipril Efficacy In Nephropathy (REIN) trial. Independent of treatment, patients with phosphate levels in the highest two quartiles progressed significantly faster either to ESRD or to a composite endpoint of doubling of serum creatinine or ESRD compared with patients with phosphate levels below the median ($P < 0.001$). Results were similar when we analyzed phosphate as a continuous variable ($P \leq 0.004$). The renoprotective effect of ramipril decreased as serum phosphate increased ($P \leq 0.008$ for interaction); this modification of the treatment effect by phosphate persisted despite adjusting for potential confounders such as GFR and urinary protein. In summary, these data suggest that phosphate is an independent risk factor for progression of renal disease among patients with proteinuric CKD, and high levels of phosphate may even attenuate the renoprotective effect of ACE inhibitors. Future trials should test whether reducing serum phosphate improves renal outcomes and optimizes the renoprotective effect of ACE inhibition.

J Am Soc Nephrol 22: 1923–1930, 2011. doi: 10.1681/ASN.2011020175

On a world scale, the prevalence of chronic kidney disease (CKD) in the general population ranges from 2.5 to 11.2%.[1] Early recognition and treatment of factors involved in renal disease onset and progression are crucial to improve outcomes of patients at risk and, at the same time, reduce the already unbearable costs for renal replacement therapy of patients who eventually progress to end stage kidney disease (ESKD). Preventing ESKD would also be lifesaving when renal replacement therapy is not available for all patients in need.

Despite early recognition and optimized treatment of established renal risk factors, however, a substantial proportion of patients with chronic nephropathies continue to progress. A plausible explanation for these discouraging observations is that known treatable risk factors account for no more than one third of the variance in renal disease progression.[2]

Pioneering experimental studies dating back to the seventies showed that reduced disposal of phosphate is one of the earliest consequences of reduced

Received February 21, 2011. Accepted June 7, 2011.

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Prof. Carmine Zoccali, CNR-IBIM, Istituto di Biomedicina, Epidemiologia Clinica e Fisiopatologia, delle Malattie Renali e dell'Ipertensione Arteriosa, c/o Euroline di Ascrizzi Vincenzo, Via Vallone Petrara n. 55/57, 89124 Reggio Calabria, Italy. Phone: 0039-0965-397010; Fax: 0039-0965-26879; E-mail: carmine.zoccali@tin.it

Copyright © 2011 by the American Society of Nephrology

nephron mass and that secondary phosphate overload play a role in the progression of renal damage by calcium-phosphate dependent and independent mechanisms.[3,4] Following studies also showed that enhanced extracellular and intracellular phosphate concentrations may engender endothelial dysfunction and oxidative stress,[5] two potential risk factors for renal damage progression. In experimental animals with reduced nephron mass, the phosphate filtration fraction progressively increases in parallel with decreasing single nephron GFR. This adaptation, aimed at maintaining phosphate balance despite a reduced filtration power, appears to be largely mediated by the upregulation of the fibroblast growth factor 23 (FGF 23).[6] This factor, however, inhibits the synthesis of vitamin D[6,7] and, by several mechanisms—including stimulated cell proliferation[6] and upregulation of the renin-angiotensin-system (RAS)[8]—may further accelerate the progression of renal damage.

To date, findings of three observational studies exploring the association between serum phosphate levels and renal outcomes[9–11] converged to indicate that phosphate might have an independent pathogenic role in the onset and progression of CKD. However, none of the analyses were run in the setting of a clinical trial with standardized guidelines for patient selection, monitoring, and treatment. Even more important, no previous study formally tested the relationship between serum phosphate levels and RAS inhibitor therapy. This is an issue of major clinical relevance since RAS inhibition is currently gold-standard therapy for patients with diabetic or nondiabetic progressive chronic nephropathies. Thus, to formally test the interactions between serum phosphate levels, renal disease progression, and RAS inhibitor therapy, we took advantage of a large cohort of patients with proteinuric chronic nephropathies randomly allocated to RAS or non-RAS inhibitor therapy and prospectively monitored, with gold standard procedures in the setting of the Ramipril Efficacy In Nephropathy (REIN) study,[12] a controlled clinical trial aimed to evaluate the renoprotective effect of ramipril therapy in CKD. Results of these analyses formed the basis of the present report.

RESULTS

Patient Characteristics

Baseline demography, clinical, and laboratory data of the 331 patients considered in the present analysis (Table 1) were similar to those of the original cohort of 352 patients included in the REIN trial[13] (data not shown). Twenty-one REIN patients were not considered because of lack of data about baseline serum phosphate levels. Of considered patients, 165 had been randomized to ramipril and 166 to placebo. Serum phosphate levels were within the normal range (2.7 to 4.6 mg/dl) in a large part of the study cohort, and only 22 (6.6%) of included patients had serum phosphate levels exceeding 4.6 mg/dl (Figure 1). In the

Table 1. Main clinical and biochemical characteristics of study patients according to serum phosphate quartiles

	1st and 2nd Phosphate Quartiles		P	3rd Phosphate Quartile		P	4th Phosphate Quartile		P
	Ramipril (*n* = 81)	Placebo (*n* = 85)		Ramipril (*n* = 48)	Placebo (*n* = 44)		Ramipril (*n* = 36)	Placebo (*n* = 37)	
Age (years)	51 ± 13	52 ± 12	0.55	48 ± 14	49 ± 16	0.92	46 ± 13	45 ± 15	0.63
Male sex, *n* (%)	68 (84%)	72 (84.7%)	0.89	36 (75%)	30 (68.2%)	0.47	28 (77.8%)	18 (50.0%)	0.01
Urinary sodium (mEq/24 h)	98 ± 39	89 ± 47	0.25	85 ± 36	88 ± 40	0.74	75 ± 27	78 ± 41	0.71
Urinary urea (mg/24 h)	1067 ± 583	984 ± 470	0.35	1017 ± 428	1010 ± 449	0.94	811 ± 392	844 ± 230	0.69
On treatment with Ca supplements, *n* (%)	2 (2.5%)	2 (2.4%)	0.96	1 (2.1%)	0 (0%)	0.34	0 (0%)	1 (2.7%)	0.32
On treatment with Vit. D analogs, *n* (%)	1 (1.2%)	1 (1.2%)	0.97	2 (4.2%)	2 (4.5%)	0.93	5 (13.9%)	2 (5.4%)	0.22
Smokers, *n* (%)	19 (23.5%)	10 (11.8%)	0.05	8 (16.7%)	9 (20.5%)	0.64	10 (27.8%)	7 (18.9%)	0.37
Diabetes, *n* (%)	4 (4.9%)	5 (5.9%)	0.79	6 (12.5%)	5 (11.4%)	0.87	3 (8.3%)	2 (5.4%)	0.62
Cholesterol (mg/dl)	241 ± 56	240 ± 53	0.96	236 ± 53	248 ± 62	0.34	258 ± 109	250 ± 55	0.71
Albumin (g/dl)	3.9 ± 0.5	3.9 ± 0.5	0.70	3.9 ± 0.5	3.9 ± 0.4	0.78	3.6 ± 1.2	3.8 ± 0.4	0.53
Glucose (mg/dl)	104 ± 26	105 ± 34	0.89	116 ± 55	110 ± 47	0.57	98 ± 23	105 ± 38	0.34
Calcium (mg/dl)	9.1 ± 1.4	9.3 ± 1.0	0.53	9.4 ± 0.5	9.4 ± 0.6	0.71	9.2 ± 0.6	9.1 ± 0.6	0.85
Phosphate (mg/dl)	2.8 ± 0.5	2.9 ± 0.4	0.07	3.8 ± 0.2	3.7 ± 0.2	0.36	4.6 ± 0.6	4.5 ± 0.4	0.15
Hemoglobin (g/dl)	14.2 ± 1.3	14.0 ± 1.7	0.55	13.7 ± 2.0	12.9 ± 1.6	0.04	12.6 ± 1.9	12.2 ± 1.7	0.38
Systolic pressure (mmHg)	145 ± 22	146 ± 17	0.86	147 ± 15	145 ± 18	0.56	146 ± 18	148 ± 19	0.69
Diastolic pressure (mmHg)	91 ± 11	91 ± 12	0.72	91 ± 14	87 ± 10	0.13	89 ± 11	91 ± 11	0.58
Mean arterial pressure (mmHg)	109 ± 14	110 ± 13	0.77	110 ± 13	107 ± 11	0.20	108 ± 12	110 ± 11	0.57
Urinary protein (g/24 h)	2.3 (1.3–3.7)	3.0 (1.5–4.3)	0.11	2.7 (1.5–4.0)	2.6 (1.4–3.9)	0.57	3.8 (2.2–7.8)	4.0 (2.0–6.6)	0.61
Iohexol GFR (ml/min/1.73m2)	52 ± 20	47 ± 17	0.05	45 ± 20	40 ± 17	0.15	35 ± 16	31 ± 13	0.27

1st and 2nd quartiles, serum phosphate <3.45 mg/dl; 3rd phosphate quartile, 3.45–4.00 mg/dl; 4th phosphate quartile, >4.00 mg/dl. Data are expressed mean ± SD or as percent frequency, as appropriate.

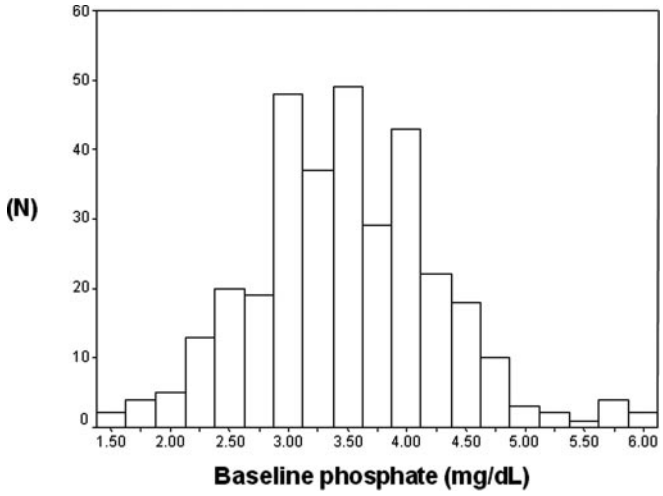

Figure 1. Distribution of baseline serum phosphate in the study population.

study group as a whole, the GFR averaged 44 ± 18 ml/min/1.73m^2 and ranged from 11 to101 ml/min/1.73 m^2. Twenty-four-hour urinary protein was <3.0 g/24h in 175 patients and exceeded 3 g/24h in the remaining 156 patients. Only a tiny minority of patients were on vitamin D supplements and/or on phosphate binders (Table 1).

Baseline demography, clinical, and laboratory data, including 24-h urinary protein, urea, and sodium excretion, were similar between treatment arms in the study group considered as a whole, as well as within different serum phosphate strata considered separately, with only few exceptions (Table 1). Smokers tended to be more frequent, serum phosphate to be lower (by approximately 0.1 mg/dl), and GFR to be higher in the ramipril than in the placebo arm among patients with serum phosphate within the range of the first two quartiles (that is, <3.45 mg/dl) (Table 1). Among patients with serum phosphate levels in the third quartile (that is, between 3.45 mg/dl and 4.00 mg/dl), hemoglobin concentration was, on average, 0.8 g/dl higher in ramipril than in those in the placebo arm, whereas among subjects in the fourth serum phosphate quartile (>4.00 mg/dl), there were more males on ramipril than or placebo (Table 1).

Phosphate and Renal Outcomes in the Study Cohort Considered as a Whole

During the follow-up period (median, 30 mo; interquartile range, 16 to 46 mo), 4 patients died, 74 progressed to ESRD, and 10 additional patients had a doubling in serum creatinine. Thus, 84 patients progressed to the combined renal end point of ESRD or doubling of serum creatinine. In the whole study cohort, the cumulative incidence of ESRD, considered as a single event or in combination with doubling of serum creatinine levels, was significantly higher ($P < 0.001$) in patients with serum phosphate levels in the third and the fourth quartile as compared with those in the first two quartiles (Figure 2, left and right panel, respectively). The differences in the progression to renal events among patients' phosphate strata remained significant ($P < 0.0025$) even after adjusting for baseline covariates—such as GFR, proteinuria, ramipril treatment, and albumin—that were identified as potential confounders for the interpretation of the effect of phosphate on study outcomes (see Concise Methods) as well as for the effect of gender and systolic BP.

Table 2), each 1-mg/dl increase in serum phosphate was associated with a 84% (shrinkage corrected: +78%) and 66% fully adjusted incremental risk for ESRD considered alone or in combination with doubling serum creatinine, respectively (Table 2).

Phosphate and Renal Outcomes According to Treatment Arm

The incidence of ESRD, considered alone or in combination with serum creatinine doubling, increased across phosphate strata in both treatment arms (Table 3). Within each phosphate stratum, the crude incidence rate of renal events was uniformly higher in patients on placebo than in those on ramipril. However, the difference in the event rates between the two treatment arms progressively decreased for increasing levels of serum phosphate (Table 3). Thus, the protective effect of ramipril therapy against progression to ESRD alone (Figure 3) or in combination with doubling of serum creatinine levels progressively vanished from the 50th phosphate percentile onward ($P \leq 0.008$ for the effect modification on both considered outcomes; see Table 3). Consistent findings were observed when serum phosphate was considered as a continuous variable (crude analyses: ESRD, phosphate-ramipril

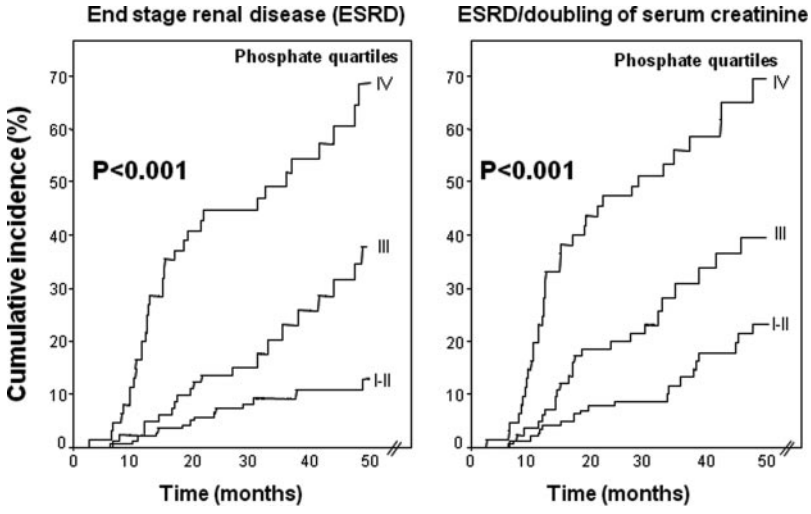

Figure 2. Cumulative incidence of ESRD alone and in combination with doubling serum creatinine in patients stratified according to serum phosphate quartiles. I/II quartile: < 3.45 mg/dl. III quartile: 3.45 to 4.00 mg/dl. IV quartile: > 4.00 mg/dl.

Table 2. Multivariate Cox regression analyses

ESRD occurrence	Crude Hazard ratio, 95% CI, and *P*-value	Adjusted for confounders, gender, and systolic BP Hazard ratio, 95% CI, and *P*-value
Phosphate (1 mg/dl)	3.30 (2.46–4.43), $P < 0.001$	1.84 (1.27–2.67), $P = 0.001$
Treatment with Ramipril (0 = no; 1 = yes)		0.48 (0.29–0.79), $P = 0.004$
Albumin (1 g/dl)		0.43 (0.26–0.73), $P = 0.002$
Hemoglobin (1 g/dl)		0.90 (0.78–1.02), $P = 0.11$
Iohexol GFR (1 ml/min/1.73m^2)		0.94 (0.92–0.96), $P < 0.001$
Urinary Protein (1 g/24 h)		1.11 (1.02–1.21), $P = 0.01$
Gender (0 = M; 1 = F)		1.71 (0.94–3.10), $P = 0.08$
Systolic pressure (1 mmHg)		1.03 (1.01–1.04), $P = 0.001$
Combined end point (ESRD/creatinine doubling)	**Crude Hazard ratio, 95% CI, and *P*-value**	**Adjusted for confounders, gender, and systolic BP Hazard ratio, 95% CI, and *P*-value**
Phosphate (1 mg/dl)	2.87 (2.18–3.77), $P < 0.001$	1.66 (1.18–2.33), $P = 0.004$
Treatment with Ramipril (0 = no; 1 = yes)		0.49 (0.31–0.79), $P = 0.003$
Albumin (1 g/dl)		0.50 (0.31–0.82), $P = 0.006$
Hemoglobin (1 g/dl)		0.92 (0.81–1.05), $P = 0.20$
Iohexol GFR (1 ml/min/1.73m^2)		0.95 (0.93–0.97), $P < 0.001$
Urinary protein (1 g/24 h)		1.11 (1.02–1.20), $P = 0.01$
Gender (0 = M; 1 = F)		1.51 (0.87–2.63), $P = 0.14$
Systolic pressure (1 mmHg)		1.02 (1.01–1.03), $P = 0.002$

The approach to multivariate Cox's regression analysis is described in detail in the Methods section. Data are expressed as hazard ratio, 95% CI, and *P*-values.

Table 3. Crude incidence rate of ESRD and (a) combined renal end point, and (b) according to serum phosphate quartiles and study arms (Ramipril versus placebo)

(a) Incidence rate of end stage renal disease (ESRD)

	Crude Incidence Rate of ESRD Occurrence *(events/100 person-years)*		*Crude Hazard Ratio, 95% CI, and *P*-value *(Ramipril versus placebo)*
	Placebo group	Ramipril group	
First two quartiles (<3.45 g/dl)	6.9 (4.0–11.2)	0.42 (0.01–2.4)	0.13 (0.04–0.39), $P < 0.0001$
Third quartile (3.45–4.00 mg/dl)	13.8 (7.6–23.2)	6.5 (3.0–12.3)	0.32 (0.18–0.59), $P < 0.001$
Fourth quartile (> 4.00 mg/dl)	26.8 (16.1–41.7)	20.7 (11.6–34.2)	0.82 (0.44–1.55), $P = 0.54$
			P for effect modification = 0.008

(b) Incidence rate of the combined renal end point (ESRD and doubling of serum creatinine)

	Crude Incidence Rate of Renal Outcomes *(events/100 person-years)*		*Crude Hazard ratio, 95% CI, and *P*-value *(Ramipril versus placebo)*
	Placebo group	Ramipril group	
First two quartiles (<3.45 g/dl)	8.8 (5.3–13.7)	1.3 (0.3–3.8)	0.15 (0.06–0.39), $P < 0.0001$
Third quartile (3.45–4.00 mg/dl)	18.6 (10.8–29.7)	6.7 (3.1–12.7)	0.37 (0.22–0.62), $P < 0.001$
Fourth quartile (> 4.00 mg/dl)	27.9 (16.8–43.8)	25.2 (14.7–40.4)	0.90 (0.49–1.66), $P = 0.73$
			P for effect modification = 0.004

Data are incidence rate and 95% confidence intervals.
*The crude hazard ratios of Ramipril treatment for study outcomes across serum phosphate quartiles were derived by Cox models including Ramipril treatment, serum phosphate strata, and their interaction term.

interaction, $P = 0.003$; ESRD/doubling of serum creatinine, phosphate-ramipril interaction, $P = 0.004$; fully adjusted analyses: ESRD, phosphate-ramipril interaction, $P = 0.022$; ESRD/doubling of serum creatinine, phosphate-ramipril interaction, $P = 0.028$). The protective effect against ESRD events achieved by ramipril *versus* placebo decreased from 87% to 68%, and to 18%, from the lowest to the middle, and the highest, serum phosphate strata, respectively (Figure 3). Data adjustment for gender, systolic BP, and potential confounders, as well as for the shrinkage correction factor, did not materially change the strength of these relationships (see Table 4 and box in Figure 3). A similar trend was observed when the combined end point was considered (Table 3), and, again, data correction for the shrinkage factor did not affect the strength of the ramipril-serum phosphate interaction (data not shown). Consistently, the adjusted hazard ratio between treatment arms for ESRD, considered alone or in combination with doubling serum creatinine, was maximally significant in the lowest phosphate stratum. The significance was virtually unmodified in the middle stratum and was lost in the highest one (Figure 3).

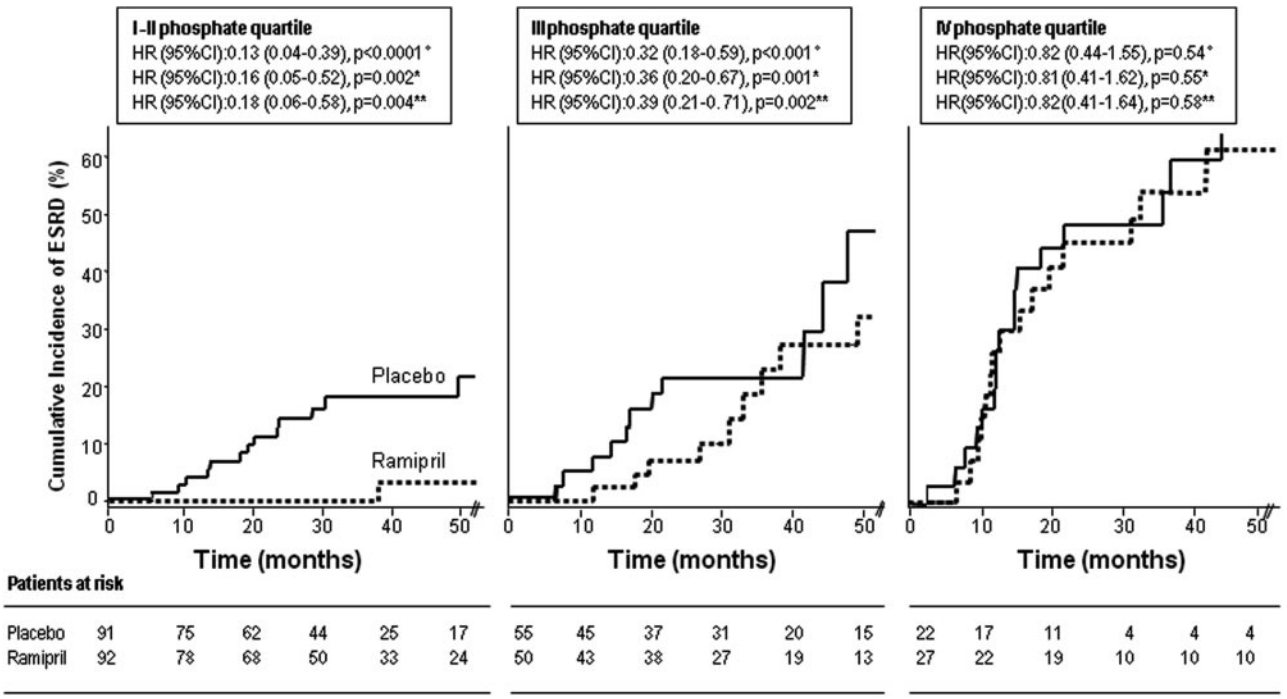

Figure 3. Effect modification of serum phosphate on the efficacy of ramipril for reducing the incidence rate of ESRD. Data are expressed as hazard ratio, 95% confidence interval, and *P*-value (see box). °Crude data. *Data adjusted for all variables listed in Table 4. **Shrinkage corrected.[24]

Table 4. Interaction analysis between Ramipril treatment and serum phosphate strata for (a) ESRD alone and (b) in combination to doubling of serum creatinine levels

(a) End stage renal disease (ESRD)	
	Adjusted for Confounders, Gender, and Systolic BP Hazard ratio, 95% CI, and *P*-value
Treatment with Ramipril (0 = no; 1 = yes)	*P* for interaction = 0.034
Phosphate strata [1 = I/II quartile; 2 = III quartile; 3 = IV quartile]	
Treatment * phosphate strata	
Albumin (1 g/dl)	0.46 (0.27–0.77), $P = 0.003$
Hemoglobin (1 g/dl)	0.89 (0.78–1.02), $P = 0.10$
Iohexol GFR (1 ml/min/1.73m^2)	0.94 (0.92–0.96), $P < 0.001$
Urinary protein (1 g/24 h)	1.13 (1.04–1.23), $P = 0.003$
Gender (0 = M; 1 = F)	1.56 (0.85–2.85), $P = 0.15$
Systolic pressure (1 mmHg)	1.03 (1.01–1.04), $P < 0.001$
(b) ESRD/doubling of serum creatinine	
	Adjusted for confounders, gender and systolic BP Hazard ratio, 95% CI, and *P*-value
Treatment with Ramipril (0 = no; 1 = yes)	*P* for interaction = 0.021
Phosphate strata [1 = I/II quartile; 2 = III quartile; 3 = IV quartile]	
Treatment * phosphate strata	
Albumin (1 g/dl)	0.53 (0.33–0.88), $P = 0.01$
Hemoglobin (1 g/dl)	0.91 (0.80–1.04), $P = 0.17$
Iohexol GFR (1 ml/min/1.73m^2)	0.95 (0.93–0.96), $P < 0.001$
Urinary protein (1 g/24 h)	1.12 (1.04–1.21), $P = 0.003$
Gender (0 = M; 1 = F)	1.34 (0.76–2.36), $P = 0.23$
Systolic pressure (1 mmHg)	1.02 (1.01–1.04), $P = 0.001$

Data are expressed as hazard ratio, 95% CI, and *P*-values.

The modification achieved by serum phosphate strata on the protective effect of ramipril therapy against progression to ESRD, considered alone or in combination with doubling of serum creatinine levels, did not vary across GFR ($P = 0.70$ and $P = 0.69$, respectively) and proteinuria ($P = 0.45$ and $P = 0.41$, respectively) levels. The effect modification of serum phosphate also did

not change when urinary sodium, urinary urea, calculated protein intake, and age were forced into Cox's models (data not shown).

DISCUSSION

In this *post hoc* analysis of outcome data retrieved from the cohort of patients with chronic proteinuric nephropathies included in the REIN trial,[12] we found that relatively higher serum phosphate levels at inclusion, even within the normal reference range, were associated with an incremental risk of progression to a renal event, either when ESRD was considered as a single end point or when it was considered in combination with doubling of serum creatinine levels. Moreover, serum phosphate levels were associated with a progressively decreasing protective effect of ramipril therapy against renal disease progression, to the point that the benefit of ACE inhibition was almost fully lost among patients with serum phosphate levels exceeding 4.5 mg/dl. Adjustment for potential confounders—including proteinuria; GFR, as measured by plasma Iohexol clearance; and other renal risk factors—did not appreciably modify these associations. Importantly, previous evidences from patients included in the REIN study demonstrated that the largest number of ESRD events saved by ramipril therapy was observed in the tertile with the lowest GFR at inclusion.[14] Thus, the reduced protective effect of ramipril against progression to ESRD observed in patients with higher serum phosphate level is not explained by more severe renal insufficiency. Overall, these findings are in keeping with the hypothesis that phosphate plays a role in renal disease progression and that excess phosphate exposure may limit or even blunt the renoprotective effect of ACE inhibitor therapy in this population.

The association between phosphate and the risk of progression of CKD was noted in previous observational studies.[9–11] In REIN we found that, independently of other risk factors, just 1-mg/dl increase in serum phosphate level was associated with a 85% excess risk for progression to ESRD and with a 65% excess risk for the combined end point. Phosphate is a strong stimulus for FGF-23 secretion. Stimulation of the FGF receptor common to all members of the FGF family increases the production of the converting enzyme,[15] which may activate the RAS. Conceivably, this might facilitate the onset and progression of kidney damage in subjects at risk and, at the same time, might overwhelm the effects of RAS inhibitors on angiotensin II production and activity, in particular at tissue level. Direct[5] and FGF23-mediated inhibitory effects of phosphate on nitric oxide (NO) production might also limit or prevent the effects of ACE inhibitor therapy mediated by NO activation. Along this line, it is worth mentioning that, in patients with CKD, plasma FGF23 levels are inversely related with NO-dependent vasodilation[16] and predict progression to ESRD.[17] On the other hand, the coreceptor of FGF23, Klotho—a well recognized renoprotective factor[18–19] that attenuates angiotensin-II-induced renal damage[20]—is downregulated at an early stage in CKD patients. Klotho −/− mice suffer premature aging, and this phenotype may be corrected with a low phosphate diet.[21] Thus, it can also be hypothesized that lower phosphate in CKD patients with reduced Klotho may mitigate the progression of renal damage and ameliorate the response to ACE inhibition.

Strengths and Limitations

Our study has limitations. First, it is a *post hoc* analysis of a clinical trial. Even although we adjusted the analysis for potential confounders, including baseline GFR, as measured by iohexol plasma clearance, and proteinuria, the possibility of residual confounding cannot be excluded. The fact that patient characteristics were very similar between treatment arms within phosphate subgroups and that main outcome data were confirmed in multivariable models is in keeping with the hypothesis that phosphate is a risk factor for progressive renal disease. Another limitation is that the REIN enrolled only Caucasian patients with nondiabetic CKD. Further studies are needed to assess whether the present findings can be generalized to non-Caucasians and to subjects with diabetic renal disease and/or to nonproteinuric nephropathies. It should also be noted that, although we adjusted our analysis for urinary sodium and urea, we did not measure phosphate excretion, which is the best clinical indicator of phosphate load. Furthermore, due to the lack of a specific bio-bank, we could not measure either FGF-23 or 1,25 vitamin D and PTH in the REIN study. Thus, pathways mediating the interactions between phosphate balance and renal disease progression need further investigation in *ad hoc* trials and mechanistic studies.

Conclusions

These *post hoc* analyses of REIN data consistently showed that phosphate is an independent risk factor for renal disease progression and that a high phosphate burden may appreciably limit or even blunt the renoprotective effect of ACE inhibitor therapy in patients with proteinuric chronic nephropathies. These findings suggest that serum phosphate might be a specific target for renoprotective therapy in CKD patients and provide the background for randomized clinical trials to formally test whether reducing phosphate exposure by restricted dietary intake and/or concomitant treatment with phosphate binding agents may serve to optimize the renoprotective effect of ACE inhibition in this population.

CONCISE METHODS

The REIN study was a prospective, randomized, placebo-controlled, multicenter clinical trial showing that treatment with the ACE inhibitor ramipril (at the target maintenance dose of 5 mg daily) significantly reduced proteinuria, compared with placebo in 352 patients with chronic progressive nephropathies, an effect that was achieved at comparable BP control between treatment arms, and that, in the long term, translated into slower GFR decline, as assessed by serial GFR measurements by the iohexol plasma clearance technique, and halved risk of progression to ESRD.[12]

In brief, study participants were normotensive (arterial pressure <140/90 mmHg without antihypertensive therapy) or hypertensive patients of both sexes, aged 18 to 70 yr, with creatinine clearance in the range 20 to 70 ml/min/1.73m^2 and persistent proteinuria (>1 g/24h for at least 3 mo before inclusion). After 1-mo placebo, run-in patients were randomized to ramipril or placebo and followed up for an average time of 30 mo (range: 0.2 to 76 mo). All considered laboratory parameters were measured by standardized procedures at local laboratories of involved centers, with the only exception of the GFR that was centrally measured by the iohexol plasma clearance technique at the laboratories of the Clinical Research Center Aldo & Cele Daccò of the Mario Negri Institute for Pharmacologic Research that coordinated and monitored all of the activities of the trial. The trial was approved by the ethical committees of all involved centers, and all of the included subjects provided a written informed consent to study participation according to the Declaration of Helsinki guidelines.

The calculated protein intake was assessed by using the standard formula: [urine urea + (weight$_{(kg)}$* 0.031)]*6.25.[22]

The aim of this *post hoc* analysis of the REIN trial was dual: (1) evaluating the relationship between serum phosphate levels at inclusion and the incidence of renal events throughout the whole observation period, and (2) assessing whether the protective effect of ramipril therapy against progression to a renal event was affected by serum phosphate.

Statistical Analyses

Main outcome variables were ESRD considered as a single end point or in combination with doubling of baseline serum creatinine *versus* baseline. Serum phosphate was tested both as a continuous variable and as categorized into quartiles, that is, below 50th percentile (combined 1st and 2nd quartiles), between the 50th and 75th percentiles (3rd quartile), and above the 75th percentile (4th quartile).

Comparisons between groups were made by the *t* test, Mann-Whitney test, or the chi-square test, as appropriate. The predictive value of serum phosphate for the incidence rate of renal events (principal effect of serum phosphate), as well as the effect modification of this anion on the efficacy of ramipril (secondary effect of serum phosphate) for reducing study outcomes (effect modification Cox regression models), was investigated by crude and multivariate Cox regression analyses (time to the first event analysis). Multivariate models investigating the principal effect of serum phosphate included this anion and variables that met criteria to be confounders,[22] that is, variables that (1) were related ($P < 0.20$) with both exposure (serum phosphate) and study outcomes, (2) were not an effect of exposure, and (3) were not in the causal pathway between the exposure and study outcomes. Because the established role of male sex and high BP in renal disease progression, all analyses were also adjusted for gender and systolic BP. By the same approach, we also identified confounders for investigating the effect modification by serum phosphate on the efficacy of ramipril for reducing the study outcomes. To this aim, we stratified the study population according to categories of serum phosphate (<50th percentile, 50th-75th percentile, and >75th percentile) and study arms. For both analyses (main effect and effect modification by serum phosphate), a common set of confounders were identified and included in the multivariate models. The proportionality assumption of Cox models was tested by the analysis of Schoenfeld residuals, and no violation was found. The multivariate Cox regression model for the combined renal end point (ESRD and doubling of serum creatinine) was of adequate statistical power (at least 10 events for each variable in the models) and a shrinkage method[24] was applied for adjusting for the same risk factors in the analysis of the main end point (ESRD). The shrinkage method is recommended in relatively small studies to avoid overfitting when the analysis is adjusted for a number of covariates exceeding the standard rule (one variable in the model for every 10 events). Relative risks between treatment arms were described by hazard ratios (HR) and their 95% confidence intervals (CI). The HRs of ramipril treatment across serum phosphate values were calculated by the linear combination method. Data were expressed as mean ± SD (normally distributed data), median, and interquartile range (non-normally distributed data), or as percent values, as appropriate. A *P*-value less than 0.05 was considered to indicate statistical significance.

All calculations were made using standard statistical packages (SPSS for Windows Version 9.0.1, Chicago, IL; STATA 9 for Windows, College Station, TX).

ACKNOWLEDGMENTS

The organization of the REIN study is as follows.

Principal investigators: G. Remuzzi, G. Tognoni.

Study coordinator: P. Ruggenenti.

* *Investigators and institutions:* N. Bossini, B.F. Viola, F. Scolari, R. Maiorca (Divisione di Nefrologia e Dialisi, Spedali Civili, Brescia); F. Cofano, G. Fellin, G. D'Amico (Divisione di Nefrologia e Dialisi, Ospedale Provinciale S Carlo Borromeo, Milano); D. Dissegna, A. Brendolan, G. La Greca (Divisione di Nefrologia e Dialisi, Ospedale S Bortolo, Vicenza); A. Feriozzi, E. Ancarani (Divisione di Nefrologia e Dialisi, Ospedale Grande di Viterbo, Viterbo); E. Gandini, I. D'Amato, A. Giangrande (Unitá Operativa di Nefrologia e Dialisi, Ospedale Provinciale, Busto Arsizio); G. Giannico, O. Vitale, C. Manno, F.P. Schena (Divisione di Nefrologia e Dialisi, Policlinico, Bari); A. Mazzi, G. Garini, A. Borghetti (Istituto di Clinica Medica e Nefrologia, Parma); R. Pisoni, L. Mosconi, T. Bertani (Divisione di Nefrologia e Dialisi, Ospedali Riuniti, Bergamo); F. Scanferla, G. Bazzato (Divisione di Nefrologia e Dialisi Ospedale Provinciale Umberto I, Mestre); E. Oliva, C. Zoccali (Divisione di Nefrologia Centro di Fisiologia Clinica del Consiglio Nazionale delle Ricerche, Reggio Calabria); G. Toti, S. Sisca, Q. Maggiore (Divisione di Nefrologia e Dialisi, Unitá Sanitaria Locale Zona 10H, Bagno a Ripoli); E. Pignone, R. Boero, G. Piccoli (Divisione di Nefrologia e Dialisi, Ospedale Zonale Giovanni Bosco, Torino); R. Piperno, A. Rosati, M. Salvadori (Unitá Operativa di Nefrologia e Dialisi, Ospedale Regionale Careggi-Monna Tessa, Firenze).

Database and statistical analyses: B. Ene Iordache, A. Remuzzi, A. Perna, R. Benini, L. Tammuzzo (Istituto di Ricerche Farmacologiche Mario Negri).

Laboratory measurements: F. Gaspari, F. Arnoldi, I. Ciocca, O. Signorini, S. Ferrari, D. Gritti, A. Roggeri, L. Del Priore, D. Cattaneo, N. Stucchi (Istituto di Ricerche Farmacologiche Mario Negri).

Independent adjudicating panel: L. Migone (chairman), E. Marubini (statistician), A. Del Favero, G. Ideo, E. Geraci, U. Loi.

This paper was partly supported by funding obtained within the framework of SYSKID (Systems Biology towards Novel Chronic Kidney Disease Diagnosis and Treatment), a project which was awarded with an European Union grant (FP7).

DISCLOSURES

None.

REFERENCES

1. Zhang QL, Rothenbacher D: Prevalence of chronic kidney disease in population-based studies: Systematic review. *BMC Public Health* 8: 117, 2008
2. Hunsicker LG, Adler S, Caggiula A, England BK, Greene T, Kusek JW, Rogers NL, Teschan PE: Predictors of the progression of renal disease in the Modification of Diet in Renal Disease Study. *Kidney Int* 51: 1908–1919, 1997
3. Ibels LS, Alfrey AC, Haut L, Huffer WE: Preservation of function in experimental renal disease by dietary restriction of phosphate. *N Engl J Med* 298: 122–126, 1978
4. Neves KR, Graciolli FG, dos Reis LM, Pasqualucci CA, Moysés RM, Jorgetti V: Adverse effects of hyperphosphatemia on myocardial hypertrophy, renal function, and bone in rats with renal failure. *Kidney Int* 66: 2237–2244, 2004
5. Shuto E, Taketani Y, Tanaka R, Harada N, Isshiki M, Sato M, Nashiki K, Amo K, Yamamoto H, Higashi Y, Nakaya Y, Takeda E: Dietary phosphorus acutely impairs endothelial function. *J Am Soc Nephrol* 20: 1504–1512, 2009
6. Seiler S, Heine GH, Fliser D: Clinical relevance of FGF23 in chronic kidney disease. *Kidney Int* 114: S34–S42, 2009
7. Zisman AL, Wolf M: Recent advances in the rapidly evolving field of fibroblast growth factor 23 in chronic kidney disease. *Curr Opin Nephrol Hypertens* 19: 335–342, 2010
8. Zhou C, Lu F, Cao K, Xu D, Goltzman D, Miao D: Calcium-independent and 1,25(OH)2D3-dependent regulation of the renin-angiotensin system in 1alpha-hydroxylase knockout mice. *Kidney Int* 74: 170–179, 2008
9. Norris KC, Greene T, Kopple J, Lea J, Lewis J, Lipkowitz M, Miller P, Richardson A, Rostand S, Wang X, Appel LJ: Baseline predictors of renal disease progression in the African American Study of Hypertension and Kidney Disease. *J Am Soc Nephrol* 17: 2928–2936, 2006
10. Schwarz S, Trivedi BK, Kalantar-Zadeh K, Kovesdy CP: Association of disorders in mineral metabolism with progression of chronic kidney disease. *Clin J Am Soc Nephrol* 1: 825–831, 2006
11. Voormolen N, Noordzij M, Grootendorst DC, Beetz I, Sijpkens YW, van Manen JG, Boeschoten EW, Huisman RM, Krediet RT, Dekker FW, PREPARE study group: High plasma phosphate as a risk factor for decline in renal function and mortality in pre-dialysis patients. *Nephrol Dial Transplant* 22: 2909–2916, 2007
12. The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia). Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. *Lancet* 349: 1857–1863, 1997
13. Ruggenenti P, Perna A, Gherardi G, Benini R, Remuzzi G: Chronic proteinuric nephropathies: Outcomes and response to treatment in a prospective cohort of 352 patients with different patterns of renal injury. *Am J Kidney Dis* 35: 1155–1165, 2000
14. Ruggenenti P, Perna A, Remuzzi G, Gruppo Italiano di Studi Epidemiologici in Nefrologia: ACE inhibitors to prevent end-stage renal disease: when to start and why possibly never to stop: A post hoc analysis of the REIN trial results. Ramipril Efficacy in Nephropathy. *J Am Soc Nephrol* 12: 2832–2837, 2001
15. Tang L, Both K, Taylor R: ACE inhibition and fibroblast growth factor in cultured human vascular smooth muscle. *Vasc Med* 4: 129–134, 1999
16. Yilmaz MI, Sonmez A, Saglam M, Yaman H, Kilic S, Demirkaya E, Eyileten T, Caglar K, Oguz Y, Vural A, Yenicesu M, Zoccali C: FGF-23 and vascular dysfunction in patients with stage 3 and 4 chronic kidney disease. *Kidney Int* 78: 679–685, 2010
17. Fliser D, Kollerits B, Neyer U, Ankerst DP, Lhotta K, Lingenhel A, Ritz E, Kronenberg F; MMKD Study Group, Kuen E, König P, Kraatz G, Mann JF, Müller GA, Köhler H, Riegler P: Fibroblast growth factor 23 (FGF23) predicts progression of chronic kidney disease: The Mild to Moderate Kidney Disease (MMKD) Study. *J Am Soc Nephrol* 18: 2600–2608, 2007
18. Haruna Y, Kashihara N, Satoh M, Tomita N, Namikoshi T, Sasaki T, Fujimori T, Xie P, Kanwar YS: Amelioration of progressive renal injury by genetic manipulation of Klotho gene. *Proc Natl Acad Sci U S A* 104: 2331–2336, 2007
19. Sugiura H, Yoshida T, Tsuchiya K, Mitobe M, Nishimura S, Shirota S, Akiba T, Nihei H: Klotho reduces apoptosis in experimental ischaemic acute renal failure. *Nephrol Dial Transplant* 20: 2636–2645, 2005
20. Yoon HE, Ghee JY, Piao S, Song JH, Han DH, Kim S, Ohashi N, Kobori H, Kuro-o M, Yang CW: Angiotensin II blockade upregulates the expression of Klotho, the anti-ageing gene, in an experimental model of chronic cyclosporine nephropathy. *Nephrol Dial Transplant* 26: 800–813, 2011
21. Kuro OM: Phosphate and Klotho. *Kidney Int Suppl* 121: S20–S23, 2011
22. Masud T, Manatunga A, Cotsonis G, Mitch WE: The precision of estimating protein intake of patients with chronic renal failure. *Kidney Int* 62: 1750–1756, 2002
23. Jager KJ, Zoccali C, Macleod A, Dekker FW: Confounding: What it is and how to deal with it. *Kidney Int* 73: 256–260, 2008
24. Kleinbaum DG, Klein M. *Survival Analysis. A Self-Learning Text* (2nd ed.), New York, Springer, 2005

See related editorial, "Phosphate REINs in the Renoprotective Benefit of ACE Inhibition," on pages 1777–1779.