

Postdialytic Rebound of Serum Phosphorus: Pathogenetic and Clinical Insights

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Abstract. To gain insights into postdialytic rebound of serum phosphate (PDR-P), serum phosphate (P), calcium (Ca), and parathyroid hormone (PTH), levels were compared from the end of treatment (T0) to the subsequent 30 to 120 min and up to 68 hr in uremic patients who underwent with crossover modality a single session of two dialytic treatments characterized by different convective removal: standard hemodialysis (HD) and hemodiafiltration (HDF). In HDF, *versus* HD, P removal was greater (1171 ± 90 *versus* 814 ± 79 mg; $P < 0.05$) in the presence of similar predialytic P levels (6.0 ± 0.2 and 5.9 ± 0.4 mg/dl) and Kt/V (1.35 ± 0.06 and 1.34 ± 0.05); however, the serum P values at T0 did not differ (3.0 ± 0.2 *versus* 3.3 ± 0.2 mg/dl). In HDF, PDR-P was more rapid (30 min *versus* 90 min) and of a greater extent (at T120: $+69 \pm$

6% *versus* $+31 \pm 4\%$; $P < 0.0001$). The higher P levels were maintained throughout the interdialytic period. $\text{Ca} \times \text{P}$ and PTH changed in parallel. Thereafter, patients were randomized to receive either HD or HDF for 3 mo. During this period, in the presence of similar Kt/V, protein intake, and dose of phosphate binder, predialytic serum P levels diminished in HDF (from 5.8 ± 0.2 to 4.4 ± 0.3 mg/dl; $P < 0.05$), but they remained unchanged in HD. A similar pattern of changes was detected in $\text{Ca} \times \text{P}$. Therefore, PDR-P is likely dependent on the mobilization of phosphate from a deep compartment induced by the intradialytic removal of this solute. Enhancement of convective removal acutely amplifies the entity of the phenomenon but allows a better control of $\text{Ca} \times \text{P}$ homeostasis in the medium term.

Hyperphosphatemia is a major adverse effect of renal failure. It leads directly, and indirectly through hypocalcemia, to hyperparathyroidism, which is the main determining factor of renal osteodystrophy (1). Moreover, hyperphosphatemia affects the survival of the dialytic population independently from the level of parathyroid hormone (PTH). In these patients, a value of serum phosphate (P) greater than 6.5 mg/dl is *per se* associated with increased (27 to 56%) relative risk of death (to convert mg/dl to mmol/L, use the following conversion factors: for P, divide by 3.098; for Ca, divide by 4.008); this is likely due to the increment in the calcium-phosphate product ($\text{Ca} \times \text{P}$) with the consequent development of metastatic calcifications at the cardiac level (2–6). The control of hyperphosphatemia therefore represents a cornerstone in the management of dialyzed patients.

To date, the preventive and/or therapeutic interventions have substantially failed; about 50% of patients show predialytic values of P higher than 6.5 mg/dl, and 19% have values greater

than 7.9 mg/dl (2,7). Conversely, the need to diminish the target limits of P and $\text{Ca} \times \text{P}$ down to 5.5 mg/dl and $55 \text{ mg}^2/\text{dl}^2$ ($4.43 \text{ mmol}^2/\text{L}^2$), respectively, has been recently highlighted to diminish morbidity and mortality rates in dialyzed patients (8).

Prevention of hyperphosphatemia is based on dietary phosphate restriction and reduction of intestinal absorption of phosphorus; however, both the therapeutic approaches are weakened by major drawbacks. The former is impractical because it requires the lowering of protein intake below 1.0 to 1.2 g/kg per day, that is, the minimum level indicated to avoid malnutrition (9,10). On the other hand, the phosphate binders commonly used in clinical practice may induce either skeletal, hematopoietic, and neurologic side effects (aluminum-based drugs) or hypercalcemia (calcium salt compounds) (9,11–14). A more suitable option will probably be represented by the new generation of calcium- and aluminum-free agents (15,16).

The dialytic treatment is the additional therapeutic tool aimed at controlling uremia-related hyperphosphatemia; however, effectiveness is limited by insufficient phosphate removal (9). A further important limitation is represented by the postdialytic rebound (PDR) of this solute, that is, the rapid increase of P during the initial few hours after the end of treatment. The extent of PDR of P (PDR-P) can be large enough to allow restoration of the predialysis P level within 4 to 12 h after the end of the dialytic session (17–22). On this regard, we have

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recently shown that the PDR-P is actually greater than that of urea and potassium (23).

Despite the clinical interest on the postdialytic rebound of P, the pathogenesis and the impact on calcium-phosphorus homeostasis are undefined. In particular, it has been recently proposed to enhance the dialytic removal of phosphate by means of hemodiafiltration, a dialytic technique characterized by a greater convective transport of solutes (20,24). Nevertheless, no study has specifically evaluated the impact of an elevated removal of phosphate on the postdialytic rebound of this solute; this is a critical point, because the entity of rebound for other solutes is proportionate to the amount subtracted during hemodialysis (25). Furthermore, even the chronic effects of hemodiafiltration on serum P levels have not been specifically examined.

In the present work, we have studied uremic patients treated, in random order and with crossover modality, by a single session of standard bicarbonate hemodialysis (HD) and hemodiafiltration (HDF). The aim of this acute study was to evaluate the pathogenetic mechanism(s) of the postdialytic rebound of phosphorus, and the effects on Ca \times P and PTH levels in the long interdialytic period. The same patients have been then randomized to either HD or HDF treatment and followed for the subsequent 3 mo. This chronic study aimed to verify the medium-term efficacy of the convective treatment in controlling hyperphosphatemia.

Materials and Methods

Patients

To select patients with stable clinical conditions, the following inclusion criteria were applied: adult anuric patients (urine output ≤ 200 ml in the long interdialytic interval) who were treated with HD in the last 6 mo did not have any acute illness and showed constancy (coefficient of variation, $\leq 30\%$) of the routine laboratory parameters measured each month (including plasma P and Ca levels after the long interdialytic interval < 7.0 and < 11.0 mg/dl, respectively, and plasma bicarbonate > 18 mmol/L); absence of clinical or laboratory signs of severe secondary hyperparathyroidism in the last 12 mo (pruritus, bone fractures, anemia unresponsive to erythropoietin therapy, and plasma levels of PTH > 1000 pg/ml); absence of diabetes mellitus, heart failure (New York Heart Association class III to IV); or advanced liver disease and peripheral or pulmonary edema.

We therefore studied, after attainment of informed consent, 12 patients (7 men, 5 women) with a mean age of 53 ± 4 yr (range, 29 to 71 yr). The underlying renal disease was primary glomerulonephritis in six patients, nephroangiosclerosis in three, and polycystic disease in three. Before the study, the patients had been dialyzed for 60 ± 10 mo (range, 12 to 110 mo). They had been regularly treated three times a week with HD for 4 h with a delivered Kt/V dose of at least 1.2 in the last 3 mo, calculated by the Daurgirdas' second generation equation (26).

All patients were constantly kept on a diet containing 30 to 35 kcal/kg body wt and 1.2 g/kg body wt of protein (50% of high biologic value). Two weeks before each experimental dialytic session, the adherence to the prescribed dietary intakes was verified by means of patient-recorded food diaries and assessment of protein nitrogen appearance (PNA).

Patients received the following medications at a dose kept constant throughout the study: antihypertensive drugs (2 patients taking cal-

cium antagonists and 2 treated with clonidine), erythropoietin (11 patients with a mean weekly dose of 6000 ± 780 U), and H2-antagonists (7 patients; 0.5 to 1.0 μg of calcitriol at the end of dialytic treatment). Calcium carbonate was used as a phosphate binder in all patients (mean dosage, 2.8 ± 0.5 g/d).

Acute Study

The study was a randomized, single-blind, crossover trial in the same group of 12 patients of a single session of two different dialytic treatments performed in the last session of the week, that is, before the long interdialytic interval: soft HDF (a dialytic procedure characterized by combined diffusive and convective fluxes, reinfusion in post-dilution of bicarbonate solution, and an ultrafiltration rate of 25 to 50 ml/min) and standard bicarbonate HD. Patients underwent the other treatment 2 wk after the first session. During the 2-wk interval, patients were treated with HD.

The modalities of the two dialytic sessions were planned to achieve, within the same time of treatment (4 h), similar values of dry weight and a Kt/V dose of 1.2 to 1.3. We used the same artificial system equipped with automatic device for determining the ultrafiltration rate (Integra; Hospal, Bologna, Italy, or System 1000; Drake Willok-Althin, Rome, Italy), same membrane (polysulfone 1.8 m², F8; Fresenius, Palazzo Pignano, Italy, or PMMA 2.0 m², Filtryzer B3–2; Toray-Hoecsht, Milan, Italy), and same blood flow rate (315 to 345 ml/min) and dialysate flow rate (500 ml/min). The dialysate composition did not differ: 39 mmol/L bicarbonate, 4.0 mmol/L acetate, 1.5 mmol/L calcium, 0.5 mmol/L magnesium, 5.6 mmol/L glucose, 2.0 mmol/L potassium. In HDF, the composition of replacement fluid was as follows: 145 mmol/L Na⁺, 100 mmol/L Cl⁻, and 45 mmol/L HCO₃⁻. In both HDF and HD, dialysate temperature and dialysate sodium concentration were kept constant (36.0°C and 143 mmol/L, respectively).

During and immediately after the dialytic session, we verified whether patients developed significant episodes of systemic hypotension, defined as a decrease of systolic arterial pressure of more than 20 mmHg or below 100 mmHg with respect to baseline.

Blood samples were obtained before the treatment, at the end (T0), after 30, 60, 90, and 120 min, and at 24, 48, and 68 h. During the entire dialysis session and in the subsequent 2 h, patients remained in bed and no food or beverage was allowed. Body weight, BP, and pulse rate were recorded hourly during the treatment and at each experimental time (from T0 to T68 h) after the dialysis session. Mean BP was calculated as the sum of one third of systolic BP and two thirds of diastolic BP.

At each experimental time, we measured hematocrit, serum levels of phosphate, calcium, potassium, sodium, total protein, urea nitrogen (BUN), glucose, bicarbonate, and pH, insulin, and intact parathyroid hormone (PTH). The PNA value, normalized for actual body weight, was calculated from the urea nitrogen appearance during the interdialytic interval from T0 to T68 (27). The samples were obtained from arteriovenous fistula through the outflow needle to limit the number of vascular punctures. The predialysis sample was obtained before the infusion of saline or heparin and before starting the blood pump, and the postdialysis sample (T0) was drawn after 2 min of maintaining a low blood flow rate (50 ml/min) in the absence of dialysate flow to minimize cardiopulmonary blood recirculation. During both dialytic treatments, the whole effluent dialysate amount was collected into a high-capacity box placed on a plate balance.

Chronic Study

After the acute study was completed, all patients continued to dialyze by means of standard HD for 2 wk. Thereafter, patients were

randomized to receive either HD ($n = 6$) or HDF ($n = 6$) in the subsequent 3 mo. Dialytic modalities and medications were the same as the acute study and were kept constant during the follow-up period. Predialytic and postdialytic (T0) blood samples were obtained with the same modalities as the acute study in the last session of the week, that is, before the long interdialytic interval. Basal data were obtained in the last session of standard HD before randomization (month 0). Thereafter, HD and HDF patients were studied at the end of each month of follow-up (months 1, 2, and 3). Blood samples were analyzed to assess predialytic P and Ca levels and the Kt/V value of the dialytic session. Patients were asked to maintain both amount and quality of food prescribed in the acute study and to fill out the dietary diaries. Compliance to diet prescription was monitored by measuring PNA and checking the dietary diaries during the interdialytic interval preceding the dialytic session.

Intraerythrocyte Solutes

In the acute study, we measured the intraerythrocyte concentration of phosphate (ery[P]), potassium (ery[K]), and urea (ery[urea]) before the dialytic session, at the end of it (T0), and after 120 min. Immediately after each blood sample was drawn, erythrocytes were separated from anticoagulated blood by centrifugation at 3500 rpm for 5 min and processed within 1 h for solutes determinations, as previously reported (23,28). Briefly, 1 ml of erythrocytes was washed with 1 ml of isoosmotic 10 mM Tris and 140 mM choline chloride washing solution to remove residual plasma cations. Thereafter, 1 ml of the washed cell suspension was added to 1 ml of 5 mM hypotonic Tris and 1 mM ethylenediaminetetraacetic acid (EDTA) solution and stored for 24 h at 4°C to obtain the complete lysis of erythrocytes. Samples were centrifuged at 3500 rpm for 5 min to remove cell membranes and, to avoid hemolysate interferences, deproteinized with 5% trichloroacetic acid solution for phosphorus or 10% Zn-sulfate and 0.75 N Na-hydroxy solution for urea nitrogen. The supernatant was analyzed with an IL-213 flame photometer (Instrumentation Laboratories, Walpole, MA) for potassium and with enzymatic methods for phosphate (Diacron, Grosseto, Italia) and for urea nitrogen (Sigma-Aldrich, Milano, Italy). All the measurements were obtained in triplicate with a coefficient of variation $\leq 1\%$.

The mean corpuscular volume (MCV) of red cells was also assessed from the ratio between hematocrit (determined by ALC hematocrit centrifuge 4203; ALC Int., Milano, Italy) and the number of red cells (determined by the analyzer H2 System Technicon; Bayer, Leverkusen, Germany), as described previously (23).

To assess the changes of intracellular phosphorus independently from the variation of MCV, we calculated the absolute amount of phosphate contained in red cells by dividing ery[P] by MCV.

Analytic Determinations

The levels of potassium and sodium were assessed by flame photometer (Beckman Instruments, Inc, Fullertone, CA) in triplicate (the coefficient of variation was always $\leq 1\%$); urea nitrogen, phosphate, calcium, total protein, and glucose were measured using an autoanalyzer (Olympus AU 560; Olympus Italia, Segrate-Milano, Italy). All the samples for determination of serum phosphate and calcium levels were measured at the same time in triplicate with a coefficient of variation $\leq 1\%$. Blood pH and bicarbonate levels were analyzed by an automatic hemogas-analyzer (ABL 625; Radiometer Copenhagen, De Mori, Italy). PTH and insulin levels were assessed by standard radioimmunoassay (Sorin, Saluggia, Italy).

The amount of phosphorus removed (P_R) by the treatment was determined according to the following formula: $P_R = V_D \times P_D$,

where V_D is the volume of collected spent dialysate and P_D is the phosphorus concentration in collected dialysate. P_D was calculated as the mean of the measurements obtained in three distinct aliquots of dialysate.

All the samples of dialysate were stored and analyzed at the same time after completion of the acute studies. To measure P_D , we used a photometric UV test for inorganic phosphorus (OSR 6222; Olympus, Hamburg, Germany). The dialysate samples were preliminarily concentrated by threefold increase of the sample volumes to have all the P_D values falling within the linearity range limits of the test (1 to 20 mg/dl) (22). The test principle is based on the reaction of phosphate with molybdate in strong acidic medium to form a complex. The absorbance of this complex in the near UV is directly proportional to the phosphate concentration. The coefficient of variation was 1.0% (range, 0.3 to 1.6%) and 0.9% (range, 0.3 to 2.1%) for P_D collected in HDF and HD, respectively. Five different amounts of P standards in water (9.530, 4.765, 2.383, 1.191, and 0.596 mg/dl) were analyzed in triplicate to evaluate the test linearity. The method showed adequate linearity within the test range of 0.596 to 9.530 ($r = 0.9998$; $P < 0.0001$). The recovery was calculated by adding to a dialysate pool sample five different amounts of P standards in water (9.530, 4.765, 2.383, 1.191, and 0.596 mg/dl). The recovery at the four concentrations of P added was 98%, 99%, 96%, 98%, and 101%. The evaluation of stability of the method over time was obtained by analysis of the five P standards (at the above specified concentrations) performed at the beginning and at the end of the analytical session. The results are expressed as percentage of coefficient of variation: 0.2%, 0.9%, 0.5%, 0.4%, and 0.5%, respectively.

Statistical Analyses

All the values are reported as mean \pm SEM. Intergroup comparisons were made by two-tailed t test for unpaired data, and intragroup comparisons were made by ANOVA for repeated measures and then by the Newman-Keuls *post hoc* test. A two-tailed $P < 0.05$ was considered statistically significant.

Results

The study group was characterized by steadiness of predialytic serum P levels, as testified by a coefficient of variation $< 15\%$ (range, 7.5 to 13.5%) in the previous 6 mo. Table 1 describes the dialytic outcome of the two treatments. The two techniques were associated with different values of ultrafiltration rate (range, 25 to 35 ml/min in HDF; 10 to 15 ml/min in HD). As prescribed, HDF and HD did not differ in treatment time and delivered dialytic dose. The reduction of extracellular volume was similar, as testified by the comparable values in both the decrement of body weight and the increment of hematocrit and serum total protein. Albumin levels changed proportionally to the value of total protein and resulted similarly in the two treatment modalities at each point of the study. BP slightly diminished by the same extent during HDF and HD, and no significant hypotensive episode was detected.

The dialytic removal of phosphorus resulted differently in the two procedures, being approximately 44% greater in HDF (Table 1). On the contrary, no difference was detected in the potassium removal. During the interdialytic period, the protein intake, evaluated by measuring PNA in the T0 to T68 h interval, was comparable (1.20 ± 0.20 and 1.23 ± 0.25 g/kg body wt per day in HDF and HD, respectively). As assessed by

Table 1. Indexes of volume state and dialysis efficiency in uremic patients before and at the end of soft hemodiafiltration and standard hemodialysis^a

Parameter	HDF		HD	
	Pre	T0	Pre	T0
Treatment time (min)		238 ± 13		237 ± 14
Intradialytic weight loss (kg)		3.0 ± 0.3		2.9 ± 0.2
Body weight (kg)	71.3 ± 3.4 ^b	68.3 ± 3.5	71.4 ± 3.4 ^b	68.5 ± 3.3
MAP (mmHg)	103 ± 2.2 ^b	101 ± 3.5	103 ± 2.6 ^b	101 ± 2.9
Hematocrit (%)	32.9 ± 0.8 ^b	37.0 ± 1.2	34.4 ± 1.0 ^b	38.0 ± 1.0
Serum total protein (g/dl)	7.10 ± 0.2 ^b	8.48 ± 0.2	7.12 ± 0.2 ^b	8.43 ± 0.2
Kt/V		1.35 ± 0.06		1.34 ± 0.05
K removal (mmol)		76 ± 9		79 ± 11
P removal (mg)		1171 ± 90 ^c		814 ± 79

^a Values are mean ± SEM. Pre, predialysis; T0, end of session; HDF, hemodiafiltration; HD, stand hemodialysis; MAP, mean arterial pressure; K, potassium; P, phosphate. Phosphate removal in mg can be converted to mmol by dividing by 30.98.

^b *P* < 0.05 versus T0; ^c *P* < 0.05 versus HD.

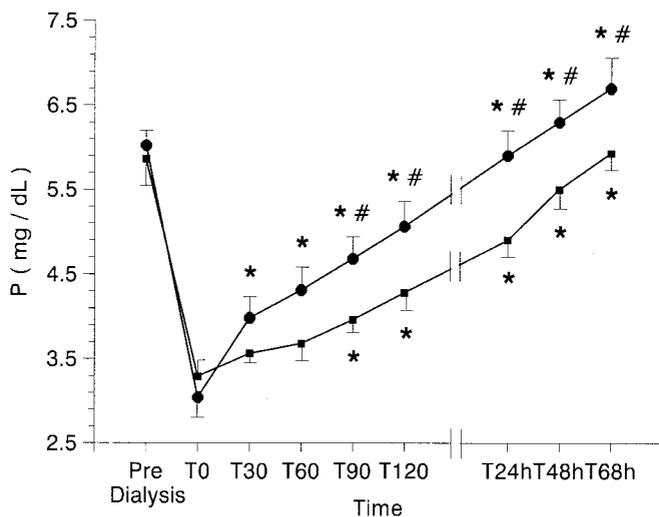


Figure 1. Serum phosphate (P) before (predialysis), at the end (T0), and 30 min to 68 h after bicarbonate dialysis (—■—, hemodialysis [HD]) and soft hemodiafiltration (—●—, hemodiafiltration [HDF]). **P* < 0.05 versus T0; #*P* < 0.05 versus HD. Phosphate concentration in mg/dl can be converted to mmol/L values by dividing by 3.098.

dietary diaries, both amount and quality of ingested food did not differ between HD and HDF.

The profile of serum P levels is depicted in Figure 1. The predialytic values of serum P were comparable in HDF and HD (6.0 ± 0.2 and 5.9 ± 0.4 mg/dl, with the difference that averaged 0.15 mg/dl). The intradialytic decline of P (Pre to T0) was only numerically higher in HDF (−50 ± 3% versus −42 ± 3% in HD; *P* = 0.098), with overlapping values of serum P at T0 (3.0 ± 0.2 and 3.3 ± 0.2 mg/dl, respectively). In contrast, the PDR of phosphorus was markedly different. A significant increment of P with respect to baseline (T0) became evident earlier in HDF (30 min) than in HD (90 min) (Figure 1). The extent of PDR was constantly greater in HDF. The increment

of P level 120 min after the end of the session was +69 ± 6% in HDF and +31 ± 4% in HD (*P* < 0.0001). Such a difference persisted throughout the entire interdialytic period, with the final value at T68 h being 6.7 ± 0.4 and 5.9 ± 0.2 mg/dl, respectively (*P* < 0.05). The P levels at T120 significantly correlated with the intradialytic removal of phosphate (*r* = 0.487; *P* < 0.02).

At variance with phosphorus, no difference was detected in the postdialytic profile of plasma potassium (K) (Figure 2, top panel). Indeed, the two treatments induced a comparable intradialytic decrement of K (−36 ± 3% in HDF; −35 ± 2% in HD), associated with a similar PDR. Similarly, no specific effect of the type of treatment was observed in the variation of blood urea nitrogen (BUN) (Figure 2, bottom panel).

During the treatment, Ca levels significantly increased (from 9.8 ± 0.3 to 10.5 ± 0.2 mg/dl in HDF and from 9.9 ± 0.2 to 10.4 ± 0.2 mg/dl in HD), with a parallel reduction in the levels of PTH (−52 ± 6% in HDF; −51 ± 5% in HD). In the next 2 h, Ca levels did not vary, being 10.2 ± 0.2 mg/dl in HDF and 10.1 ± 0.2 mg/dl in HD at T120. In contrast, PTH rapidly increased, returning to values not different from the predialytic level in both HDF and HD (Figure 3, top panel). Notably, the PTH values at T120 significantly differed in the two treatments, with the absolute increase from T0 being more pronounced after HDF; the percent increase of PTH at T120 was, in fact, +186 ± 35% in HDF and +69 ± 14% in HD (*P* < 0.005).

The changes of Ca × P are depicted in the bottom panel of Figure 3. The treatment-induced reduction of Ca × P was similar in HDF and HD (−46 ± 4% and −39 ± 3%). However, the greater PDR of phosphorus in HDF resulted in a larger increase of Ca × P at T120 with respect to HD (+64 ± 6% versus +25 ± 5%; *P* < 0.05). Such a difference persisted up to the end of the observation period; at T68 h, the value was 64 ± 3 mg²/dl² in HDF and 55 ± 2 mg²/dl² in HD (*P* < 0.05).

Table 2 depicts the changes of the factors playing a secondary role in the control of P. During the dialytic treatment, blood

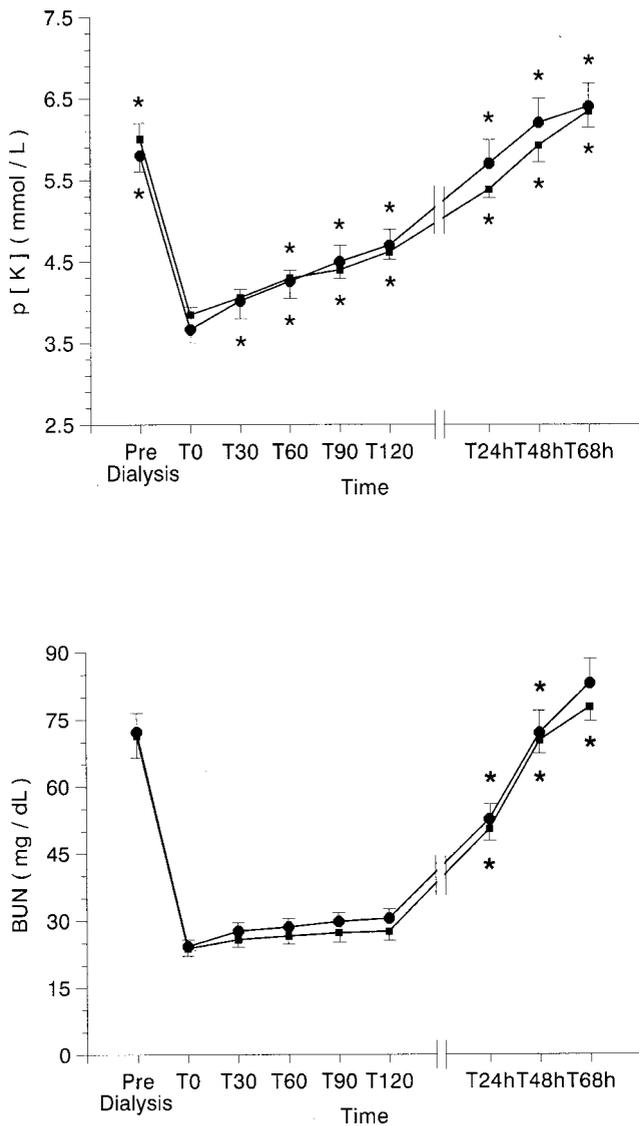


Figure 2. Plasma K⁺ (top panel) and blood urea nitrogen (bottom panel) before (predialysis), at the end (T0), and 30 min to 68 h after bicarbonate dialysis (—■—, HD) and soft hemodiafiltration (—●—, HDF). *P < 0.05 versus T0. Blood urea nitrogen concentration in mg/dl can be converted to mmol/L values by multiplying by 0.357.

pH and bicarbonate levels, which were comparable at baseline, similarly increased in HDF and HD. Both parameters did not vary in the postdialysis period; however, the absolute increment of bicarbonate from Pre to T120 was greater in HDF (+6.3 ± 0.4 mmol/L) than in HD (+5.1 ± 0.4 mmol/L; P < 0.02). Plasma sodium and insulin levels were unaffected by both dialytic modalities.

The intracellular/extracellular (I/E) redistribution of water was evaluated by determining the MCV of red cells. MCV significantly decreased by the same extent during HDF (from 89.4 ± 1.8 to 87.9 ± 0.8 fl) and HD (from 90.1 ± 1.2 to 88.4 ± 0.9 fl). MCV continued to decrease after treatment; at T120, the value was on average 87.2 ± 0.7 fl and 87.8 ± 1.0 fl in HDF and HD, respectively.

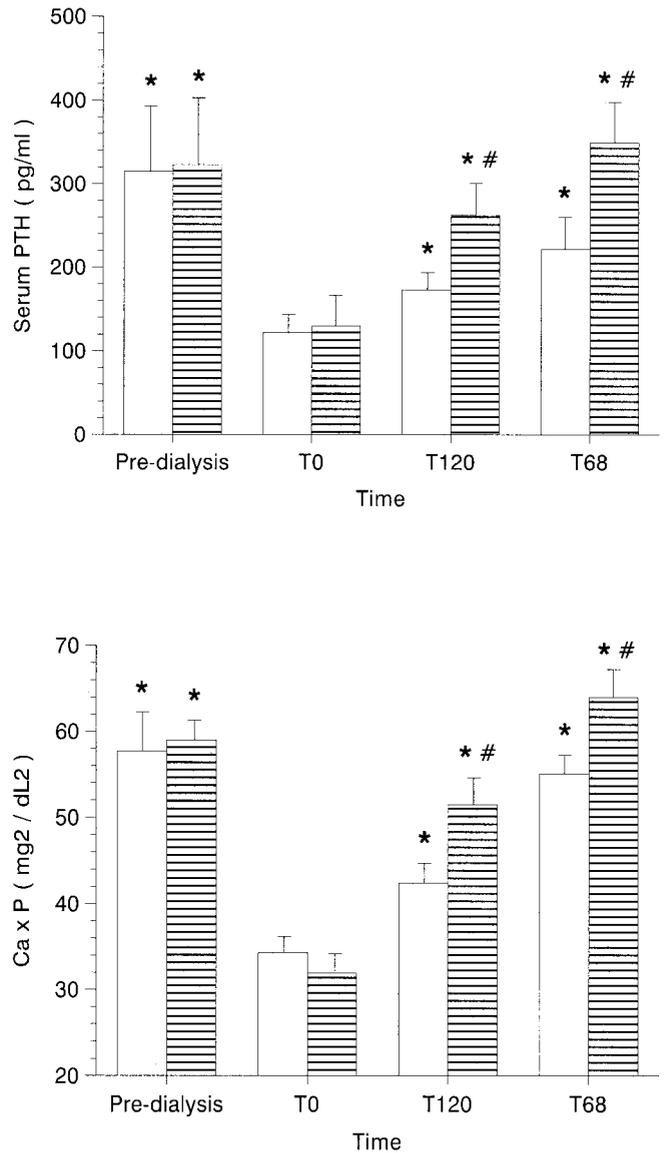


Figure 3. Serum parathyroid hormone (PTH) levels (top panel) and calcium-phosphorus product (Ca × P) (bottom panel) before (predialysis), at the end (T0), and 120 min (T120) and 68 h (T68) after bicarbonate dialysis (□, HD) and soft hemodiafiltration (▨, HDF). *P < 0.05 versus T0; #P < 0.05 versus HD. Phosphate and calcium concentration in mg/dl can be converted to mmol/L values by dividing the former by 3.098 and the latter by 4.008.

As reported in Table 3, the I/E ratio of phosphorus at the end of treatment was significantly greater in HDF than in HD. This was the result of the entity of the combined changes in intracellular and extracellular concentrations of phosphorus during treatment; at the end of HDF, as compared with HD, the intraerythrocyte concentration of phosphorus was numerically higher (588 ± 15 versus 541 ± 24 mg/L red cells) and serum phosphate was slightly lower (3.04 ± 0.23 versus 3.29 ± 0.20 mg/dl). In the next 2 h, this ratio significantly diminished to values not different between the two modalities, but the percent decrement was greater after HDF (−48 ± 2%) than after HD

Table 2. Blood pH and plasma levels of bicarbonate, sodium, and insulin before and 120 min after the end of soft hemodiafiltration and standard hemodialysis^a

Parameter	HDF			HD		
	Pre	T0	T120	Pre	T0	T120
pH	7.349 ± 0.010	7.448 ± 0.010	7.451 ± 0.010	7.342 ± 0.011	7.444 ± 0.010	7.449 ± 0.009
HCO ₃ ⁻ (mmol/L)	22.7 ± 0.7 ^b	28.0 ± 0.7	29.0 ± 0.7	22.4 ± 0.9 ^b	26.5 ± 0.9	27.5 ± 0.8
Na ⁺ (mmol/L)	141 ± 1 ^b	145 ± 1	146 ± 1	141 ± 1 ^b	144 ± 1	145 ± 1
Insulin (mU/L)	26.0 ± 5.0	24.5 ± 5.4	28.0 ± 6.0	25.9 ± 5.9	25.7 ± 5.3	28.2 ± 6.1

^a Values are mean ± SEM. HCO₃⁻^b *P* < 0.05 versus T0.**Table 3.** Intraerythrocyte/extracellular ratio of phosphorus, potassium, and urea before and immediately and 120 min after the end of soft hemodiafiltration and standard hemodialysis^a

Parameter	HDF			HD		
	Pre	T0	T120	Pre	T0	T120
I/E [P]	9.2 ± 0.3 ^b	19.6 ± 0.5 ^c	10.1 ± 0.2 ^b	9.0 ± 0.4 ^b	16.4 ± 0.7	10.5 ± 0.3 ^b
I/E [K]	16.2 ± 0.3 ^b	26.7 ± 0.5	20.7 ± 0.2 ^b	15.9 ± 0.2 ^b	25.4 ± 0.2	20.9 ± 0.3 ^b
I/E [urea]	0.77 ± 0.02 ^b	1.04 ± 0.06	0.80 ± 0.02 ^b	0.75 ± 0.02 ^b	1.09 ± 0.06	0.85 ± 0.02 ^b

^a Values are mean ± SEM. I/E [P], intraerythrocyte/extracellular ratio of phosphorus; I/E [K], I/E of potassium; I/E [urea], I/E of urea.^b *P* < 0.05 versus T0.^c *P* < 0.05 versus HD.

(−34 ± 2%; *P* < 0.0001). Similarly, the decrement of the absolute amount of phosphate per red cell in postdialysis was more pronounced in HDF; the cell loss of phosphate was, in fact, almost the double in HDF (−7.9 ± 1.2 fg) with respect to HD (−4.5 ± 0.6 fg; *P* < 0.05), but the final (T120) values overlapped. At variance with phosphorus, the I/E ratios of potassium and urea did not significantly differ between HDF and HD in predialysis, at T0, or in postdialysis (Table 3).

Table 4 depicts the pattern of variation of P levels in HD and HDF patients over the 3-mo period of observation after the acute study. The Kt/V values, which were similar to the values measured in the acute study, did not differ between HD and HDF groups throughout follow-up. The nutrient intake was also similar, as indicated by the comparable values of PNA measured in the interval before the dialytic sessions. Furthermore, as assessed by the dietary diaries, patients maintained unvaried amount and quality of food during the 3-mo period of follow-up, with no difference between the two groups. Finally, the dose of phosphate binder (calcium carbonate) did not differ between HD and HDF patients at baseline (HDF, 2.6 ± 0.6 g/d; HD, 2.9 ± 0.4) and was kept unchanged during the entire period of follow-up.

Under these conditions, the group of patients treated with HDF was characterized by a 24% decline of predialytic P levels. On the contrary, no change was observed in the group randomized to receive HD. The improvement of P control in HDF became significant in the second month of follow-up, with no further amelioration in the third month. Similarly, Ca

× P diminished in HDF (from 53.5 ± 2.1 to 39.9 ± 3.3 mg²/dl²; *P* < 0.05), and it remained unchanged in HD (from 51.3 ± 4.4 to 53.8 ± 3.5 mg²/dl²).

Discussion

To improve phosphate balance in uremic patients, a great effort has recently been made to enhance the dialytic removal of this solute. Conventional hemodialysis by allowing a weekly removal of about 2500 mg is largely inadequate for elimination of the total amount of phosphorus absorbed in 1 wk (4000 to 5000 mg) from a standard protein intake (9,19). Therefore, standard HD of 4-hr length is not efficacious if not associated with some dietary restriction and therapy with phosphate binders. Previous reports have evidenced the enhancement of phosphorus removal in course of hemodiafiltration secondary to the greater convective flux (20,24). In the current study, HDF allowed a removal of phosphate that was about 44% higher than that obtained in HD. In contrast, no difference in the removal of potassium and urea was observed; the dialytic clearance of these solutes, in fact, is mainly influenced by diffusive transport (24,25,29).

The intradialytic decline of phosphatemia did not significantly change between HD and HDF despite the different removal. Several other studies have demonstrated that the mass removal of phosphate, as opposed to urea, cannot be predicted by the intradialytic variation of serum P (18,20,21,24). Indeed, P levels decrease rapidly in the first 2 h of dialytic treatment and remain constant or rise slightly thereafter, independently

Table 4. Predialytic values of serum phosphate, Kt/V, protein nitrogen appearance, and intradialytic weight loss before (basal) and 1 to 3 mo after randomization of patients to either standard hemodialysis ($n = 6$) or soft hemodiafiltration ($n = 6$)

Parameter	HDF			HD				
	Basal	Mo 1	Mo 2	Mo 3	Basal	Mo 1	Mo 2	Mo 3
Serum P (mg/dl)	5.8 ± 0.2	4.9 ± 0.2 ^b	4.4 ± 0.2 ^{b,c}	4.4 ± 0.3 ^{b,c}	5.7 ± 0.4	5.4 ± 0.4	5.8 ± 0.5	5.7 ± 0.4
Kt/V	1.40 ± 0.06	1.43 ± 0.06	1.49 ± 0.11	1.44 ± 0.08	1.41 ± 0.10	1.44 ± 0.06	1.45 ± 0.10	1.41 ± 0.10
PNA (g/kg per d)	1.23 ± 0.05	1.30 ± 0.10	1.29 ± 0.09	1.26 ± 0.07	1.25 ± 0.08	1.25 ± 0.07	1.30 ± 0.08	1.24 ± 0.07
Weight loss (kg)	3.2 ± 0.3	3.5 ± 0.2	3.4 ± 0.3	3.5 ± 0.4	3.5 ± 0.3	3.6 ± 0.3	3.6 ± 0.4	3.7 ± 0.3

^a Values are mean ± SEM. PNA, protein nitrogen appearance. Phosphate concentration in mg/dL can be converted to mmol/L value by dividing by 3.098.

^b $P < 0.05$ versus basal.

^c $P < 0.05$ versus HD.

from dialyzer blood flow, length of the session, and Ca fluxes, with the rate of decrement being correlated only with the predialytic P level and dialyzer surface area (17–21,29,30). The constancy of P values in the second part of a dialysis session, despite ongoing removal, is consistent with net addition of phosphate to the extracellular fluid (ECF) from a deep extraskeletal compartment, that is, cells and/or soft tissues, when serum P levels are lowered enough by dialytic removal (18,19,21,31,32).

The current study reinforces this hypothesis and adds novel information by providing evidence that the enhancement of convective removal mobilizes phosphorus from the deep phosphate pool with no net loss of phosphorus from the cellular store. We did not assess the intradialytic phosphate kinetics; however, in agreement with DeSoi and Umans (21), we may speculate that the enhanced P removal in course of HDF was associated with a major decrement of serum P levels in the first part of dialytic session. This phenomenon, leading to a greater mobilization of phosphate from the deep compartment to ECF, resulted in a more pronounced rise of serum P levels in the second part of treatment, which accounts for the absence of difference in the serum P levels at the end of HD and HDF. Furthermore, this study allows the examination of the isolated effect of convective flux on phosphate removal, that is, independently from the other influencing factors, such as the predialytic P level (29,33) and dialyzer surface area (29); indeed, the initial P levels were comparable in HD and HDF, and the type of dialyzer was not modified in the two treatments.

The larger removal of phosphate obtained by the convective technique was associated with a major postdialytic rebound of P (Figure 1). After HDF, as compared with HD, the PDR-P developed promptly, with the increment of P levels being significant as soon as 30 min after the end of treatment. Furthermore, the magnitude of the phenomenon was greater in the convective treatment. The increase of P levels measured 2 h after the end of HDF was of $69 \pm 6\%$, which is a value more than doubled with respect to HD ($+31 \pm 4\%$). A similar correlation between entity of dialytic removal and extent of PDR has been previously reported for urea (25). However, urea rebound is of moderate degree and limited to the first postdialytic hour (23,25,30), but the PDR-P can be clinically significant, as indicated in the present and previous studies (17–23).

The greater PDR-P was strictly dependent on the larger intradialytic removal of this solute. In fact, we found a significant correlation between the entity of phosphate removal and the values of serum P measured 2 h after the end of treatment. The diverse pattern of changes of postdialytic P levels in HDF and HD occurred independently from the main factors known to influence the PDR of phosphorus. Zucchelli and Santoro (20) have shown that PDR of phosphorus is greater in acetate-dialysis than bicarbonate-dialysis, suggesting a role for the intradialytic changes of pH. They also showed that PDR-P is proportionate to the predialysis P value (20). In our study, both the predialytic phosphatemia and pH values did not differ in HD and HDF; however, the absolute increment of blood bicarbonate concentration was actually greater in HDF. Furthermore, insulin levels were similar in the two dialytic modalities,

therefore excluding that the PDR of phosphorus was affected by the insulin-mediated transcellular shift of phosphate (21). Interestingly, our group has recently demonstrated that higher plasma tonicity is coupled with major PDR of potassium and phosphorus because of a greater efflux from cells of potassium and phosphate by means of solvent drag mechanism of transport (23). In the present study, the dialysate sodium concentration was kept constant to avoid any influence of plasma tonicity. Consequently, in the two dialytic treatments, the intracellular to extracellular shift of water was similar, as suggested by the comparable reduction of MCV during and after HDF and HD. Finally, it has been demonstrated that the extracellular potassium removal by inducing the cell membrane hyperpolarization may induce a shift of phosphate from the cell to restore the membrane potential (32); this effect can be excluded in our study because potassium removal was similar in the two dialytic procedures. Therefore, we can reasonably hypothesize that in HDF the major flux of phosphate from the deep compartment to ECF, secondary to the enhancement of intradialytic removal, resulted in a greater increment of serum P levels from the second part of treatment up to the postdialysis period.

Transcellular shifts of solutes are involved in the pathogenesis of PDR (23); therefore, we have analyzed the I/E gradients of phosphate, potassium, and urea. At the end of HDF, the I/E gradient of phosphate was significantly higher than in HD because of the minimal reduction in serum P levels combined with a slight increment of intracellular phosphate concentration. In the 2 h after the end of session, the percent decrement of this ratio was greater in HDF, suggesting a higher efflux of phosphate from the intracellular compartment after the convective treatment. This was confirmed by the significantly greater reduction after HDF of the absolute amount of phosphate in red cell. The contribution of the transcellular shift of phosphate to the entity of PDR-P was likely minimal because both the I/E gradient and the intracellular amount diminished to values not different between the two modalities. On the contrary, the pattern of changes in the I/E gradient of urea and potassium was comparable in HDF and HD, possibly because of similar removal and minor cell compartmentalization; this finding was coupled with analogous postdialytic variation in the serum levels of both solutes in the two treatments.

At variance with previous reports (17–22), patients in our acute study were monitored throughout the entire long interdialytic period. The enhancement of PDR-P in HDF was associated with P levels that remained higher throughout the observation time, up to the next dialysis session. Consequently, the $\text{Ca} \times \text{P}$ product was also constantly higher in HDF than in HD, up to 68 h after the dialytic session. Although patients were not kept in our clinical research center during the interdialytic period, the higher P level observed in the T24 h to T68 h period after HDF was probably not related to changes of phosphorus intake; indeed, the PNA values after the two sessions were comparable in the T0 to T68 h interval, and food diaries showed no variation of both amount and quality of nutrients. Of note, the impact of a different PDR was not limited to the $\text{Ca} \times \text{P}$ product. The PTH levels were similar in

HDF and HD before and at the end of the session. However, PTH at T120 increased by a greater extent in HDF than in HD and remained higher throughout the interdialytic period (Figure 3). Such a difference was strictly dependent on the higher phosphatemia in HDF. The two dialytic modalities were, in fact, characterized by similar changes of serum calcium, and the dose of calcitriol given at the end of treatment was kept constant. Previous studies have suggested that hemodialyzed patients may have phosphorus-related changes of PTH without changes in serum calcium and calcitriol (34,35). Our data add novel information to this issue by evidencing in uremic patients, as previously demonstrated in rats by Slatopolsky *et al.* (36), that the direct stimulating effect of phosphate levels on PTH release is rapid, occurring within 2 h after the end of the session. These data, therefore, demonstrate that PDR-P is associated with acute detrimental effects on calcium-phosphorus homeostasis when patients are shifted from HD to HDF.

Conversely, beneficial effects of HDF became apparent when the patients were examined in the medium term (Table 4). In the 3-mo period of observation, we found that HDF significantly improved the control of P and $\text{Ca} \times \text{P}$ levels. This effect was independent from the delivered dialytic dose and occurred in the absence of changes in the dosage of phosphate binder and protein intake level. The decline of predialytic P levels in HDF was likely related to the progressive reduction of phosphate content in the deep compartment.

In conclusion, this study in uremic dialyzed patients suggests that (1) PDR-P is dependent on the mobilization of phosphate from a deep compartment to ECF induced by the intradialytic removal of this solute; and (2) enhancement of the convective removal of phosphate, obtained by changing treatment from standard HD to HDF, acutely amplifies the entity of the phenomenon but allows a better control of calcium-phosphorus homeostasis in the medium term.

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