Kidney International, Vol. 64 (2003), pp. 2272–2279

Circulating concentration of FGF-23 increases as renal function declines in patients with chronic kidney disease, but does not change in response to variation in phosphate intake in healthy volunteers

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Circulating concentration of FGF-23 increases as renal function declines in patients with chronic kidney disease, but does not change in response to variation in phosphate intake in healthy volunteers.

Background. Hyperphosphatemia is a risk factor for the development of several different complications of chronic kidney disease (CKD), including secondary hyperparathyroidism and cardiovascular complications, due to the formation of calcium-phosphate deposits. Fibroblast growth factor-23 (FGF-23) is a recently discovered protein that is mutated in autosomal-dominant hypophosphatemic rickets, an inherited phosphate wasting disorder, and it may represent a novel hormonal regulator of phosphate homeostasis. We therefore hypothesized that FGF-23 levels may be altered in hyperphosphatemia associated with renal failure and that its concentration changes in response to different levels of phosphate intake.

Methods. Using a two-site enzyme-linked immunosorbent assay (ELISA) detecting the C-terminal portion of FGF-23, serum concentration was measured in 20 patients with different stages of renal failure (creatinine range 155 to 724 μmol/L), in 33 patients with end-stage renal disease (ESRD) on dialysis treatment, and in 30 patients with functioning renal grafts. Furthermore, six healthy males were given oral phosphate binders in combination with low dietary phosphate intake for 2 days followed by 3 days of repletion with inorganic phosphate. FGF-23 levels were determined at multiple time points.

Results. FGF-23 serum levels were significantly elevated in CKD with a strong correlation between serum creatinine and FGF-23 concentration. Independent correlations were also seen between FGF-23 and phosphate, calcium, parathyroid hormone (PTH), and $1,25(OH)_2D_3$. No changes in serum FGF-23 levels were observed in volunteers following ingestion of oral phosphate binders/low dietary phosphate intake, which led to a decline in phosphate excretion or during the subsequent repletion with inorganic phosphate through oral phosphate and a normal diet.

Conclusion. Circulating FGF-23 was significantly elevated in patients with CKD and its concentration correlated with renal creatinine clearance. In healthy volunteers, FGF-23 levels did not change after phosphate deprivation or phosphate loading.

Key words: FGF-23, renal disease, hyperphosphatemia, phosphate loading, phosphate homeostasis.

Received for publication January 23, 2003
and in revised form March 10, 2003, and May 29, 2003
Accepted for publication July 29, 2003

Patients with chronic kidney disease (CKD) show impaired renal excretion of phosphate that leads to significant hyperphosphatemia [1]. Hyperphosphatemia is an important risk factor for the development of several complications of CKD, including formation of ectopic calcifications, cardiovascular disease, and secondary hyperparathyroidism [2]. Consequently, dietary phosphate restriction and treatment with oral phosphate binders are parts of the standard treatment regimen in these patients [1, 3].

Autosomal-dominant hypophosphatemic rickets, oncogenic osteomalacia, and X-linked hypophosphatemia are phosphate-wasting disorders that share similar clinical and biochemical characteristics, including low serum phosphate levels due to increased renal phosphate clearance and inappropriately low 1,25 dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] levels [4]. Recent findings suggest that fibroblast growth factor-23 (FGF-23), the protein mutated in autosomal-dominant hypophosphatemic rickets, may be involved in the pathogenesis of all three disorders. In autosomal-dominant hypophosphatemic rickets, three different mutations were identified at position 176 or 179 of full-length FGF-23. These mutations are thought to inhibit inactivation of biologically active FGF-23 by making the protein resistant to cleavage between position Arg179 and Ser180 and possibly other sites [5].

FGF-23 mRNA and protein are found at high levels in oncogenic osteomalacia tumors. Furthermore, recombinant FGF-23 given parenterally to rodents or

transgenic expression of this growth factor leads to hypophosphatemia, while ablation of the FGF-23 gene in mice results in significant hyperphosphatemia [6–8]. Based on these studies, it was therefore postulated that this growth factor causes, directly or indirectly, renal phosphate wasting [9, 10]. Recently, we developed a two-site enzyme-linked immunosorbent assay (ELISA) detecting the C-terminal portion of human FGF-23 protein [11]. Using this ELISA, we demonstrated that FGF-23 levels are readily detectable in healthy individuals. Furthermore, FGF-23 concentrations were markedly increased in most patients with oncogenic osteomalacia and returned to within, or close to, the normal range after surgical removal of the tumors [11]. In a significant number of patients with X-linked hypophosphatemia, circulating levels of FGF-23 were also found to be increased [11].

To further explore whether FGF-23 might be involved in the regulation of phosphate homeostasis, we measured FGF-23 concentrations in patients with renal failure who often develop severe hyperphosphatemia, if left untreated, and in normal volunteers on a low phosphate diet/phosphate binders and during replenishment with dietary phosphate.

METHODS

CKD patients

We consecutively recruited 83 patients with varying severity of renal failure. The patients were divided into three groups based on treatment and severity of renal disease: (*1*) a predialysis group consisting of 20 patients (creatinine range 155 to 724 μmol/L), (*2*) a dialysis group consisting of 33 patients treated with either continuous ambulatory peritoneal dialysis (CAPD) ($N = 9$) or standard bicarbonate hemodialysis three times a week ($N = 24$) (creatinine range 500 to 1200 μmol/L), and (*3*) a transplant group consisting of 30 patients representing a cross-section of renal transplant patients, all with a varying degree of renal graft function but generally with a better renal function than in the group of predialysis patients (creatinine range 93 to 364 μmol/L). The patients in the predialysis group and some patients in the dialysis group, who continued to have some urine output, were also treated with diuretics. Additional medications included oral phosphate binders, a phosphate restricted diet and $1,25(OH)_2$ vitamin D (Rocaltrol®; Roche, Stockholm, Sweden) or 1,25OH vitamin D (Etalpha®; Leo Pharma, Malmö, Sweden). The underlying diseases in this cohort were glomerulonephritis (37%), type 1 diabetes (20%), polycystic kidney disease (12%), interstitial nephritis, and obstructive nephropathy (10%), hypertensive nephrosclerosis (9%), type 2 diabetes (9%), and others (5%). Blood samples were collected during routine monthly visits to the outpatient clinic (for dialysis patients at midweek). The time posttransplant at which the blood was analyzed was 91 ± 14.4 months. Routine biochemical parameters were analyzed immediately while additional serum samples were frozen and stored in $-70^{\circ}C$ for subsequent analysis of FGF-23 levels.

Phosphate loading/deprivation experiment

Six males between 25 and 45 years of age with no known health problems and no medication were selected for this study. Blood samples were collected at 8:00 a.m., 12:00 a.m., 4:00 p.m., and 8:00 p.m. daily for 6 days. All morning samples were fasting samples. Day 1 contained no restrictions or medications. Days 2 and 3 contained a standardized diet with 2900 kcal per day and a total phosphate intake of approximately 870 mg per day. For this purpose a liquid diet specially prepared for patients with renal failure was used (Nepro®) (Abbott Nutrition, Stockholm, Sweden). Additionally, four tablets of phosphate binder (Renagel® 403 mg/tablet) (Genzyme, Cambridge, MA, USA) were taken three times a day. Days 4 to 6 contained a normal diet supplemented with 600 mg of inorganic phosphate four times a day. The study was approved by the local ethics committee (approval number 01-449).

Biochemical analyses

Serum and urine FGF-23 levels were measured, according to the manufacturer's instructions, using the C-terminal human FGF-23 ELISA (Immutopics, San Juan Capistrano, CA, USA). This ELISA was developed using an affinity-purified antibody raised against [Tyr-223] FGF-23(206–222) amide as capture antibody and an affinity-purified biotinylated antibody against [Tyr-224] FGF-23(225–244) amide as detection antibody; recombinant human FGF-23 generated by transfected SF9 cells was used as standard; the assay sensitivity was 3.0 RU/mL (normal range 0 to 157 RU/mL). This ELISA has been previously described in detail [11]. Dilutions of serum samples were performed in sample diluent (i.e., zero standard) provided by the manufacturer. For CKD patients, serum $1,25(OH)_2D_3$ (normal range 20 to 60 ng/L) levels were analyzed by Silab, Malmö, Sweden using liquid chromatography and a competitive radioreceptor assay from Incstar Corp., Stillwater, MN, USA, respectively. Intact parathyroid hormone (PTH) was measured by immunoradiometric assay (IRMA) (Nichols Institute, San Juan Capistrano, CA, USA) (normal range 10 to 65 ng/L) at Uppsala University Hospital. For the healthy volunteers, $1,25(OH)_2D_3$ was measured with gamma-B 1,25-$(OH)_2$-dihydroxy vitamin D kit (IDS, Boldon, England) and full-length PTH with Elecsys PTH kit (Roche Diagnostics, Mannheim, Germany) (normal range 10 to 60 ng/L) at Uppsala University Hospital. Calcium (normal range 2.20 to 2.60 mmol/L), albumin (normal range 40 to 51 g/L), phosphate (normal range 0.74 to 1.54 mmol/L),

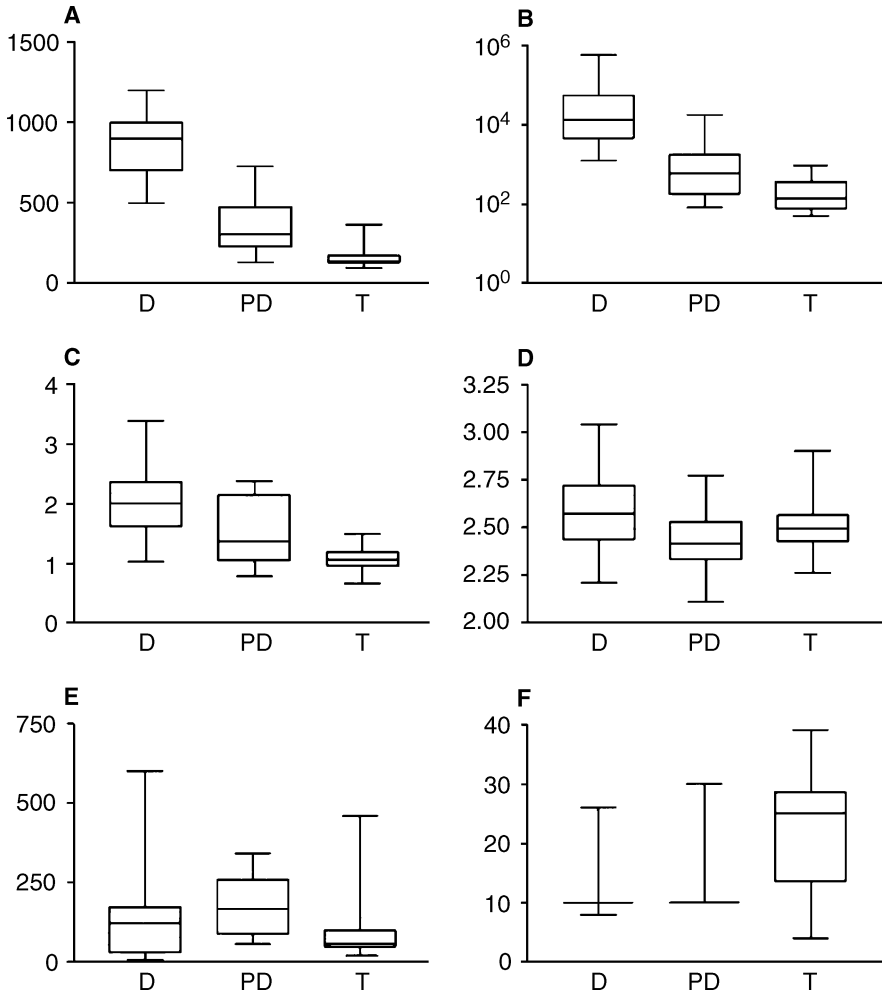

Fig. 1. The serum values of the three separate groups. (*A*) Creatinine (umol/L). (*B*) Fibroblast growth factor-23 (FGF-23) (RU/mL). Note that scales are logarithmic. (*C*) Phosphate (mmol/L). (*D*) Calcium (mmol/L). (*E*) Parathyroid hormone (PTH) (ng/L). (*F*) $1,25(OH)_2D_3$ (ng/L). Abbreviations are: D, dialysis group; PD, predialysis group; T, transplant group. The boxes represent the mean ± SEM and the vertical lines the maximum and minimum values in the population measured.

and creatinine (normal range 69 to 113 μmol/L) were measured by routine methods at the department of clinical chemistry at Uppsala University hospital.

Western blot analysis

A total of 200 μL of urine or serum from dialysis patients and healthy volunteers were immunoprecipitated with 20 μL of a rabbit antibody directed against human [Tyr-224] FGF-23(225–244) amide (the antibody has been previously described [10]) and incubated at 4°C with end-over-end rotation overnight. Then, 200 μL of protein A sepharose (Pharmacia Amersham, Uppsala, Sweden) was added and incubated for 30 minutes at room temperature. The samples were spun and rinsed twice with Tris-HCl buffer (pH 8.0) containing NaCl (150 mmol/L final concentration) and 0.01% NaN_3. Twenty microliters of the solution were then mixed with 20 μL sodium dodecyl sulfate (SDS) loading buffer for electrophoreses through 15% SDS-polyacrylamide gel electrophoresis (PAGE) minigels (Bio-Rad, Hercules, CA, USA). Subsequent Western blot analyses were performed according to standard procedures using the rabbit anti-[Tyr-223] FGF-23(206–222) amide or rabbit anti-[Tyr-224] FGF-23(225–244) amide as primary antibody as earlier described [10] (1 mg/mL; dilution 1:2000), followed by incubation with a horse radish peroxidase (HRP)-coupled goat antirabbit antibody (Dako, Dakopatt AB, Älvsjö, Sweden) [diluted 1:5000 in 5% milk in phosphate-buffered saline (PBS)] as secondary antibody. Visualization of immunoreactive human FGF-23 was performed by enhanced chemiluminescence (ECL) (Amersham, Piscataway, NJ, USA).

Statistics

The StatView SE + Graphics software package (Abacus Concepts, Inc., Berkeley, CA, USA) was used for all statistical analyses. Values below the detection limit for PTH and $1,25(OH)_2D_3$ were in calculations arbitrarily assigned the value of half of the difference between the detection limit and zero. Values are expressed as mean ± SEM unless otherwise stated.

Table 1. Relationship between fibroblast growth factor-23 (FGF-23) and other biochemical parameters

Total population	β value	R^2 value	*P* value
FGF-23			
Creatinine	94.1	0.168	=0.0001
Phosphate	44600	0.133	<0.001
Calcium	202000	0.179	<0.0001
Parathyroid hormone	255	0.129	<0.001
$1,25(OH)_2$ vitamin D_3	−731	0.006	0.495
Log FGF-23			
Creatinine	0.003	0.766	<0.0001
Phosphate	1.213	0.526	<0.0001
Calcium	2.481	0.144	<0.001
Parathyroid hormone	0.003	0.069	<0.05
$1,25(OH)_2$ vitamin D_3	−0.063	0.238	<0.0001

A simple regression model with serum values of creatinine, phosphate, calcium, PTH, and $1,25(OH)_2D_3$ as independent variables versus eigher FGF-23 or log FGF-23 as dependent variable analyzed in the entire population studied. The log FGF-23 values assumed a closer approximation to a normal distribution curve than the FGF-23 values.

RESULTS

The blood chemistry data for patients with CKD disease grouped according to treatment are presented in Figure 1. The mean creatinine levels for the predialysis group was 360 ± 170 μmol/L, for the dialysis group 850 ± 190 μmol/L, and for the renal transplant group 150 ± 50 μmol/L (Fig. 1A), reflecting the differences in renal function between the groups. The corresponding concentrations of FGF-23, phosphate, calcium, PTH, and $1,25(OH)_2D_3$ are illustrated for each group in Figure 1B through F. FGF-23 levels were markedly increased in the dialysis group (59,600 ± 20,500 RU/mL) and, albeit to a much lesser extent, in the predialysis group (2010 ± 890 RU/mL), while transplanted group showed FGF-23 concentrations that were close to the normal range (230 ± 42 RU/mL).

For the entire patient population, the FGF-23 serum levels correlated with the concentrations of creatinine ($R^2 = 0.168$; $P = 0.0001$), phosphate ($R^2 = 0.133$; $P < 0.001$), calcium ($R^2 = 0.179$; $P < 0.0001$), and PTH ($R^2 = 0.129$; $P < 0.001$), when performing a univariate regression analysis. The data are presented in Table 1. The FGF-23 values assumed a closer approximation to a standard distribution curve when transformed to logarithms and the correlations increased with creatinine ($R^2 = 0.766$; $P < 0.0001$) and phosphate ($R^2 = 0.526$; $P < 0.0001$) (Fig. 2). The log FGF-23 concentration was also positively correlated with calcium ($R^2 = 0.144$; $P < 0.001$) and PTH ($R^2 = 0.069$; $P < 0.05$) and negatively with $1,25(OH)_2D_3$ ($R^2 = 0.238$; $P < 0.0001$).

However, as the three groups were clearly distinguishable clinical entities and had different mean FGF-23 values the three groups were also analyzed separately. A simple regression model with FGF-23 as the dependent variable and creatinine, phosphate, calcium, PTH, and $1,25(OH)_2D_3$ as independent variables was used. The data are summarized in Table 2. In the dialysis group, both calcium, PTH, and $1,25(OH)_2D_3$ co-varied with FGF-23 ($P < 0.01$). In the predialysis group, phosphate was the only significant variable correlating with FGF-23 ($P < 0.05$). The log FGF-23, which even within the groups more resembled normally distributed values, correlated to creatinine ($P < 0.05$), phosphate ($P < 0.05$), calcium ($P < 0.05$), and $1,25(OH)_2D_3$ in the dialysis group. Correlations increased with creatinine ($P < 0.001$) and phosphate ($P < 0.01$) in the predialysis group compared to the absolute FGF-23 values in the same group. In the transplant group, no linear or logarithmic relationship between FGF-23 and other biochemical parameters was seen.

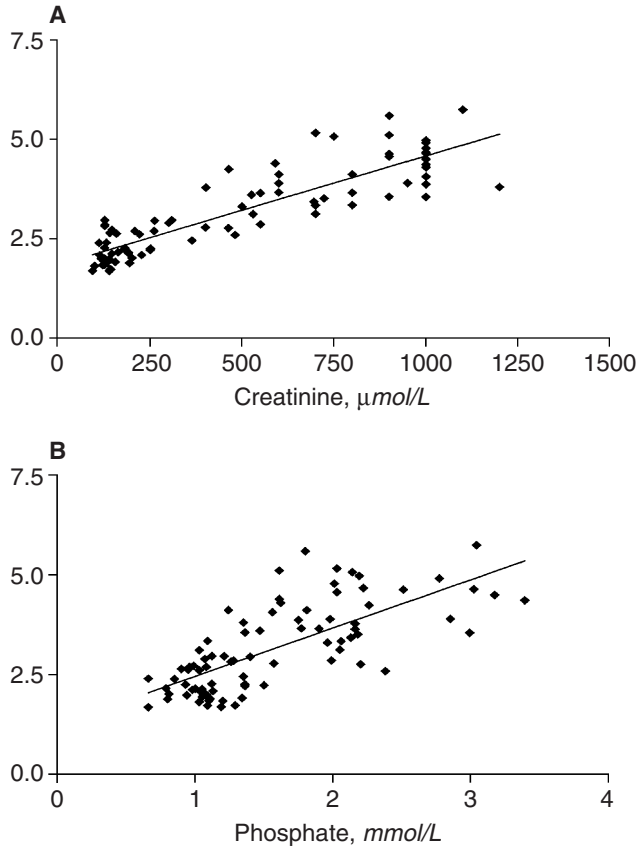

Fig. 2. The correlation for entire population of 83 patients with varying stages of renal disease between the log fibroblast growth factor-23 (FGF-23) serum concentration and creatinine (*A*) or phosphate (*B*).

To further explore the elevated circulatory levels of FGF-23 in CKD patients, we performed immunoprecipitation followed by Western blotting of serum from one CKD patient and one healthy volunteer with normal FGF-23 serum level. In the CKD patient, a protein band of approximately 32 kD was detected, corresponding to the expected molecular weight of full-length FGF-23 [6, 9, 10, 12] (Fig. 3). No lower-molecular-weight protein bands were detected. In the healthy volunteer, only a faint 32 kD band was seen.

Table 2. Relationship between fibroblast growth factor-23 (FGF-23) and other biochemical parameters

Subpopulation	β value	R^2 value	*P* value
Dialysis (FGF-23)			
Creatinine	175	0.076	0.120
Phosphate	46000	0.057	0.180
Calcium	317000	0.235	<0.01
Parathyroid hormone	417	0.224	<0.01
$1,25(OH)_2$ vitamin D_3	21700	0.269	<0.01
Predialysis (FGF-23)			
Creatinine	8.27	0.125	0.126
Phosphate	3560	0.235	<0.05
Calcium	9960	0.160	0.081
Parathyroid hormone	1.97	0.002	0.859
$1,25(OH)_2$ vitamin D_3	−112	0.029	0.485
Transplant (FGF-23)			
Creatinine	0.434	0.010	0.598
Phosphate	155	0.017	0.497
Calcium	124	0.005	0.720
Parathyroid hormone	0.301	0.012	0.564
$1,25(OH)_2$ vitamin D_3	−3.53	0.019	0.480
Dialysis (log FGF-23)			
Creatinine	0.002	0.194	<0.05
Phosphate	0.409	0.125	<0.05
Calcium	1.704	0.188	<0.05
Parathyroid hormone	0.002	0.084	0.1016
$1,25(OH)_2$ vitamin D_3	0.091	0.131	<0.05
Predialysis (log FGF-23)			
Creatinine	0.003	0.487	<0.001
Phosphate	0.754	0.417	<0.01
Calcium	1.165	0.086	0.210
Parathyroid hormone	0.002	0.082	0.2197
$1,25(OH)_2$ vitamin D_3	−0.017	0.027	0.5010
Transplant (log FGF-23)			
Creatinine	0.002	0.057	0.202
Phosphate	0.211	0.011	0.578
Calcium	−0.022	5.207E-5	0.970
Parathyroid hormone	0.001	0.046	0.253
$1,25(OH)_2$ vitamin D_3	−0.006	0.017	0.496

A simple regression model with serum values of creatinine, phosphate, calcium, PTH, and $1,25(OH)_2$ vitamin D_3 as independent variables versus FGF-23 or log FGF-23 as dependent variables analyzed in three separate groups: dialysis, preuremic, and transplant groups. Even in the separate groups, the log FGF-23 values assumed a closer approximation to a normal distribution curve than the FGF-23 values.

Increased FGF-23 levels in renal failure could be due to a decreased clearance of FGF-23 by the kidney. We therefore measured FGF-23 levels in urine from dialysis patients with severe renal disease but residual urine production. Urine levels of FGF-23 measured in four dialysis patients ranged from 750 to 10,790 RU/mL, and serial dilutions of these urine samples ran in parallel to the recombinant FGF-23 standard (data not shown). The presence of FGF-23 immunoreactivity in urine was confirmed by immunoprecipitation followed by Western blot analysis (Fig. 3). Several specific low-molecular protein bands were readily detected in urine from CKD patients but only faint bands were seen when urine from normal volunteers was immunoprecipitated.

In the second part of our study, we determined FGF-23 serum levels in six healthy males four times each day for 6 days. FGF-23 showed no clear circadian variation; the mean concentration of FGF-23 was 38 ± 8 RU/mL for the entire group. This value was close to the previous determined mean concentration of FGF-23 in a group of 141 healthy individuals [11]. Only one subject in our study showed an average FGF-23 level of 110 U/mL, which is still within 2 SD the reference range. This individual also had the highest content of urinary FGF-23 among the participants (87.6 ± 5.6 RU/mL).

The percentage of inorganic phosphate reabsorbed in the kidney in relation to total amount of phosphate filtrated (%TPR) (normal range 75% to 100%) was used to determine the efficiency of renal inorganic phosphate reabsorption. As previously observed [13] %TPR increased during phosphate deprivation and thus resulted in a marked reduction in urinary phosphate excretion; during phosphate loading %TRP was significantly reduced from baseline determined during phosphate deprivation ($P < 0.01$) when analyzed with the Mann-Whitney test. Surprisingly, no significant differences in serum or urine FGF-23 levels were observed during the study (Fig. 4A and B, respectively). However, serum levels of phosphate, PTH, and $1,25(OH)_2D_3$ did not reveal significant changes throughout the study (Fig. 4C, E, and F, respectively).

DISCUSSION

The most striking finding of the present study was the strong correlation between serum FGF-23 and creatinine levels in the CKD patient population as a whole. Recent preliminary findings indicated that experimentally induced renal failure in rats also results in a significant rise in serum FGF-23 levels [14]. A possible reason for this could be that FGF-23, or at least its C-terminal fragment, is normally cleared by the kidneys. In support of this, we show that FGF-23 immunoreactivity is present in the urine of healthy individuals and that the amounts are greatly increased in urine from CKD patients with residual urinary output. In some dialysis patients, absolute serum values of FGF-23 levels were increased, compared to healthy individuals, by more than a 1000-fold. The magnitude of this increase also argues for a decreased FGF-23 clearance in CKD. The finding that the healthy volunteer with serum FGF-23 levels at the upper end of the normal range had the highest urine content of FGF-23 also suggest renal clearance of FGF-23. In addition, we found that urine FGF-23 concentration were tenfold higher in one patient with oncogenic osteomalacia and normal renal function than in any of the healthy volunteers (data not shown). Accumulation of protein fragments were previously reported for other peptide hormones and proteins, including PTH and osteoprotegerin, and the accumulation of FGF-23 in serum may thus contribute, through yet unknown mechanisms, to the skeletal resistance to PTH and bone disease observed in chronic renal failure [15, 16].

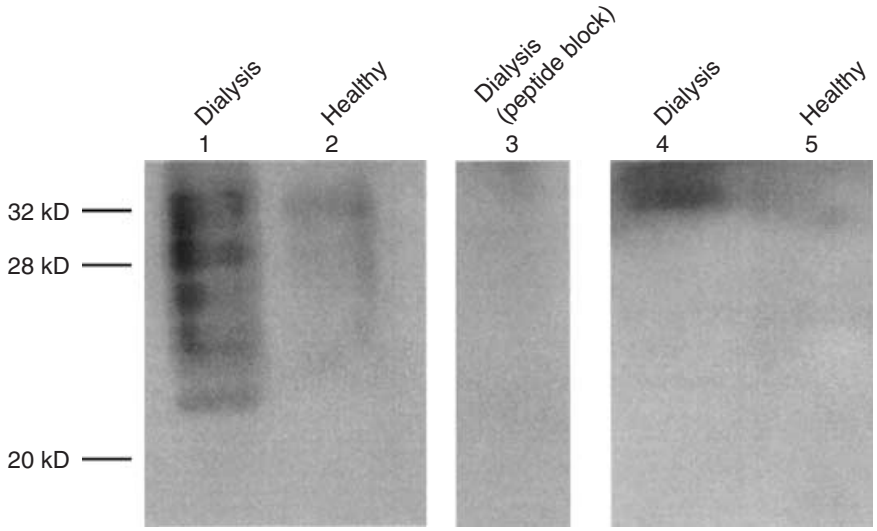

Fig. 3. Western blot analysis using urine from a dialysis patient with some residual renal function (lane 1) and a healthy volunteer (lane 2) after immunoprecipitation with rabbit anti-[Tyr-224] FGF-23(225–244) amide antibody. Using the same anti-[Tyr-224] FGF-23(225–244) amide antibody, a ≈32 kD band and several other protein bands of lower molecular weight were readily detected in urine from chronic kidney disease (CKD) patients; these bands were only faint when urine from the normal volunteer was immunoprecipitated. When the antibody for detection was preincubated with excessive amount of peptide that was used for immunization, none of these protein bands was detected (lane 3). Lanes 4 and 5 displays serum after immunoprecipitation from a CKD patient and a healthy volunteer, respectively. Only a ≈32 kD band was detected in both lanes, although much weaker in the healthy volunteer.

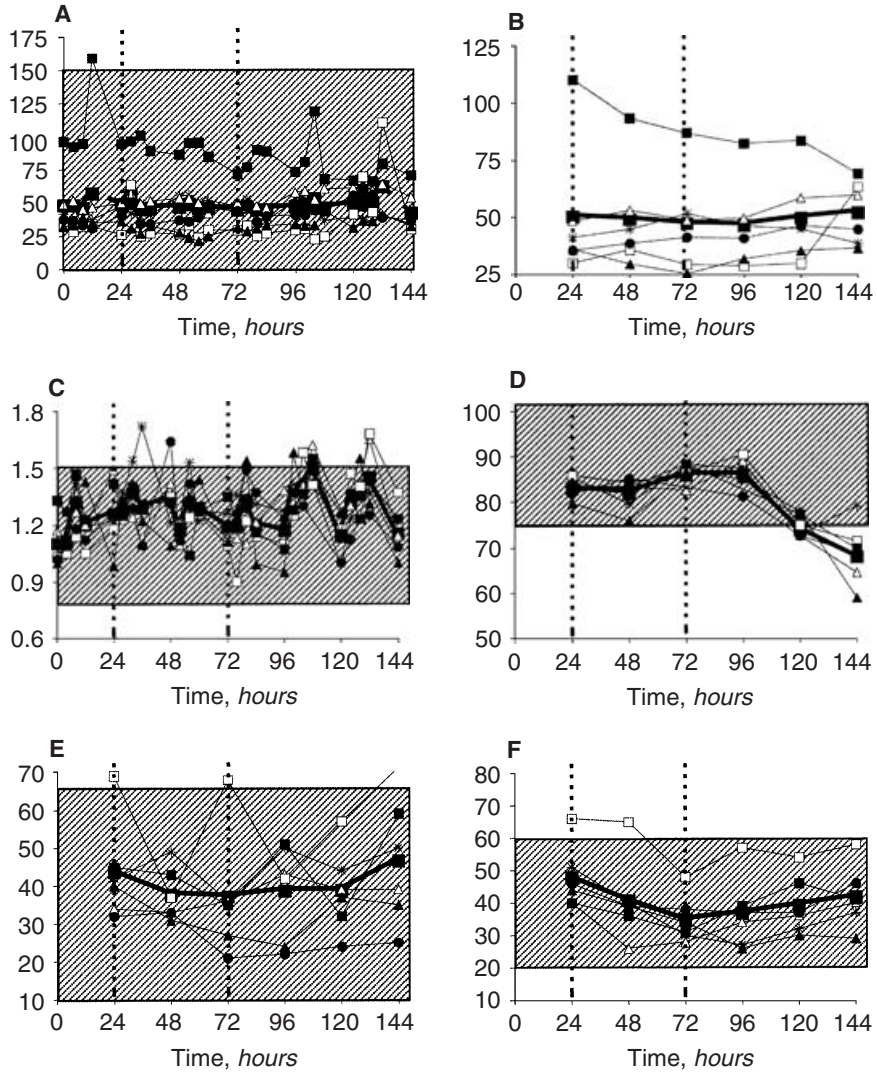

Fig. 4. Serum and urine biochemical parameters measured during the phosphate deprivation/loading experiment in six healthy volunteers marked as P1 to P6. (*A*) Fibroblast growth factor-23 (FGF-23) (RU/mL). (*B*) u-FGF-23 (RU/mL). (*C*) Phosphate (mmol/L). (*D*) Total amount of phosphate (TPR) (%). (*E*) Parathyroid hormone (PTH) (ng/L). (*F*) $1,25(OH)_2D_3$ (ng/L). FGF-23 and phosphate were measured at 8:00 a.m., 12:00 a.m., 4:00 p.m., and 8:00 p.m. throughout the experiment. u-FGF-23, %TPR, PTH, and $1,25(OH)_2D_3$ were measured every day at 8:00 a.m. The hours 0 to 24 represent the calibration period; hours 24 to 72, the phosphate deprivation phase; and hours 72 to 144, the phosphate loading phase. The dashed vertical lines indicate the time at which phosphate deprivation and phosphate loading, respectively, was started. The hatched areas represent the adult normal ranges for the different parameters.

The elevated levels of FGF-23 in renal failure could also be due to a direct physiologic response to hyperphosphatemia. This hypothesis is supported by a recent finding that hyperphosphatemia induced by dietary means causes a marked increase in circulating FGF-23 levels in rats [17]. Our finding that the predominant FGF-23 immunoreactivity in serum from a CKD patient was of the expected full-length molecular weight, suggests that the increased FGF-23 levels could partly be due to increased production of intact FGF-23 protein. However, to determine whether this mechanism contributes to the increase, it will be necessary to demonstrate increased FGF-23 production in tissue samples from CKD patients.

Given the fact that FGF-23 induces hypophosphatemia in vivo [5], we speculated that an external alteration in phosphate load would regulate the endogenous production of FGF-23. In our study, the tubular reabsorption of phosphate increased significantly during phosphate deprivation; conversely %TPR decreased following refeeding with phosphate. However, the high doses of inorganic phosphate given to our volunteers failed to trigger a rise in FGF-23 despite the significant increase in renal phosphate clearance, suggesting that intact FGF-23, or the fragments that are also measured with this assay, is not directly responsible for the acute adjustment of phosphate excretion in the kidney. However, it is possible that long-term circulating phosphate levels are the most important factor for the regulation of FGF-23 and no change in serum phosphate was seen in this study. Alternatively, a long half-life of the FGF-23 protein could obscure the effects of acute manipulations. However, recently published data suggest a short half-life of intact FGF-23 in tumor-induced osteomalacia, which does not support the latter hypothesis [12]. Another reason for not detecting changes in FGF-23 levels could be the use of an ELISA that only recognizes the C-terminal portion of FGF-23 and not exclusively the full-length protein. It has been reported that only the full-length protein is capable of inducing hypophosphatemia in vivo [5] and it is therefore possible that the active protein is indeed responsible for the change in phosphate reabsorption but that an excess of degradation products masks the effect. However, our finding, which is in agreement with previous reports [12] that biologic full-length FGF-23 constitutes the main portion of circulating FGF-23 in healthy individuals, argues against this hypothesis.

We also measured FGF-23 levels in 24-hour urine collection samples. These samples contained detectable levels of FGF-23 and there was no change in urine concentrations during the different phases of phosphate loading. Given that FGF-23 normally appears to be cleared by the kidneys and that its half-life is short one could expect to find an increased urinary concentration of intact or fragment(s) of FGF-23 if the endogenous production increased during phosphate loading. As this was not the case, our data support the contention that FGF-23 may not be regulated by acute changes in blood phosphate concentration.

In an unpublished report where healthy volunteers were placed on a high phosphate intake for 2 weeks, a small but significant increase in C-terminal FGF-23 levels was detected [19]. In our study, four of six volunteers showed their highest serum and urine FGF-23 levels during the high phosphate intake phase. This suggests that FGF-23 serum levels indeed will respond to external phosphate alterations, but that the time course for phosphate-induced changes in FGF-23 concentration is different from that chosen for our experiments and that more chronic changes in dietary phosphate are required to influence serum FGF-23 levels. Furthermore, it is also possible that patients with renal failure may be more susceptible to changes in FGF-23 in response to dietary phosphate than the healthy subjects investigated in this study. Thus it appears possible that short-term changes in phosphate intake do not lead to readily detectable alterations in FGF-23 serum concentration, but that in long-term hyperphosphatemia, as is often the case in patients with CKD, the FGF-23 production might be increased. Such a hypothesis would imply that FGF-23 is not the hormonal mechanism responsible for the rapid adjustment of renal phosphate clearance in response to change in dietary intake of phosphate.

CONCLUSION

Circulating levels of FGF-23 protein are increased in patients with impaired renal function, but in this study no regulation of FGF-23 was observed after short-term changes in dietary phosphate intake. However, these preliminary findings will require additional studies in the future, including measurement of intact FGF-23 when such an assay becomes available.

ACKNOWLEDGMENTS

We would like to thank Anna-Lena Johansson for technical assistance and Richard Zahradnik and Jeffrey Lavigne for valuable discussions. This study was supported by The Swedish Foundation for International Cooperation in Research and Higher Education.

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