Acute Effects of Very-Low-Protein Diet on FGF23 Levels: A Randomized Study

Biagio Di Iorio,* Lucia Di Micco,* Serena Torraca,* Maria Luisa Sirico,* Luigi Russo,† Andrea Pota,* Francesco Mirenghi,* and Domenico Russo†

Summary

Background and objectives High levels of fibroblast growth factor 23 are associated with mortality, CKD progression, and calcification in CKD patients. The aim of this pilot study is to assess whether a very-low-protein diet (0.3 g/kg per day) with a consequent low intake of phosphorus would reduce fibroblast growth factor 23 compared with a low-protein diet (0.6 g/kg per day) in CKD patients not yet on dialysis.

Design, setting, participants, & measurements A prospective, randomized, controlled crossover study was performed in which 32 patients were randomized into two groups. Group A (16 patients) received a very-low-protein diet (0.3 g/kg body wt per day) supplemented with ketoanalogues during the first week and a low-protein diet during the second week, and group B (16 patients) received a low-protein diet during the first week and a very-low-protein diet during the second week. Fibroblast growth factor 23,-seric, and urinary phosphate levels were measured at baseline and the end of each study period.

Results After only 1 week of the very-low-protein diet, reductions in fibroblast growth factor 23 levels (33.5%), serum phosphate (12%), and urinary phosphate (34%) with the very-low-protein diet compared with the low-protein diet were observed. Serum and urinary phosphate levels and protein intake were significant determinants of fibroblast growth factor 23 (95% confidence interval = 1.04–1.19, 1.12–1.37, and 1.51–2.23, respectively).

Conclusions A very-low-protein diet supplemented with ketoanalogues reduced fibroblast growth factor 23 levels in CKD patients not yet on dialysis.

Introduction

It is well known that alterations of calcium-phosphate metabolism that occur in CKD affect cardiovascular (CV) events, mortality, and CKD progression (1,2). Potential culprits for progression are hyperphosphatemia, secondary hyperparathyroidism (sHPT), lack of active vitamin D, and high levels of fibroblast growth factor 23 (FGF23) (3–5). This hormone is primarily secreted by osteocytes in response to dietary phosphorus overload or increase in 1,25-dihydroxyvitamin D, and it stimulates renal excretion of P, downregulates the production of 1,25-dihydroxyvitamin D, and reduces parathyroid hormone (PTH) levels (6,7). In patients with CKD, FGF23 levels are thought to increase as a compensatory response to maintain normal P balance, because the capacity for renal P excretion declines (8). Recent studies indicate that increased levels of FGF23 are associated with mortality, CKD progression, and calcification in CKD patients (9–12). These evidences suggest that early treatment of calcium-phosphate alterations may lead to improved management of CKD progression and associated CV complications. No studies have evaluated the effects of a dietetic phosphate restriction on FGF23 in CKD patients not yet on dialysis. The aim of this study is to assess whether a very-low-protein diet (0.3 g/kg per day) with a consequent low intake of phosphorus would reduce FGF23 compared with a low-protein diet (0.6 g/kg per day) in CKD patients not yet on dialysis.

Materials and Methods

Our study had a prospective, randomized, controlled crossover design; it was carried out in the CKD outpatients’ clinic of the Nephrology Division at the A. Landolfi Hospital of Solofra (Avellino, Italy). All patients gave informed consent; the study was approved by the Ethics Committee of ASL AVELLINO, Avellino (Italy). We screened 68 consecutive patients; 53 of them were eligible for the study, but only 32 agreed to participate and were enrolled from June of 2008 to June of 2010. Inclusion criteria were written informed consent, age >18 years, renal function measured with creatinine clearance <55 and >20 ml/min (three monthly consecutive measurements), at least 6 months of follow-up at our clinic, achievement of therapeutic targets according to the Best Clinical Practice (BP<130/80 mmHg,
hemoglobin $>$ 11 g/dl, serum bicarbonate levels between 20 and 24 mEq/L, total cholesterol $<$ 200 mg/dl, LDL cholesterol $<$ 100 mg/dl, triglycerides $<$ 180 mg/dl). Exclusion criteria were pregnancy, history of dialysis and/or renal transplant, diabetes, proteinuria $>$ 2 g/d, PTH $>$ 500 pg/ml, malignancies, active infectious diseases, immunosuppressive drugs given in the last year or any other drug that could interfere with mineral metabolism, rapid changes of the residual renal function, and inability to perform a urinary collection of 24 hours. During the 3-month run-in period, diet history and nutritional status of all patients were evaluated by an expert dietician, and patients who met the inclusion criteria were asked to adhere to a low-protein diet (LpD; 0.6 g/kg body wt per day). Patients were instructed on how to obtain a careful urinary collection. Urine collection was considered inaccurate and discarded if measured creatinine excretion rate was outside the 60–140% range of the value estimated according to the work by Dwyer and Kenler (13). Creatinine clearance values were confirmed three times in 3 consecutive months (a variation of 0.5% above or below the initial data was allowed); nutritional and metabolic parameters were evaluated. After the run-in period, patients were matched for age, sex, and creatinine clearance (patients were subclassified in three groups of creatinine clearance: 55–45, 44–30, and 29–20 ml/min) and randomized to groups A and B. A simple randomization list was generated by means of a computer and kept concealed with the use of numbered, opaque sealed envelopes opened in sequence by administrative staff personnel not involved in patient care. Group A (16 patients) received a very-low-protein diet (VLpD; 0.3 g/kg body wt per day) during the first week and LpD during the second week; group B (16 patients) received LpD during the first week and VLpD during the second week. There was no washout period between the two different diets.

All patients were prescribed at least 30 Kcal/kg per day of energy. VLpD diet was supplemented with a mixture of ketoanalogue and essential amino acids (Alfa Kappa; Shire Italia, Florence, Italy) administered at the dose of one pill per 5 kg body weight to maintain the neutral nitrogen balance even at the lower protein intakes, thus increasing the efficiency of nitrogen use and maintaining a good nutritional status. Each pill contained calcium keto-isoleucine (67 mg), calcium keto-leucine (101 mg), calcium keto-alanine (68 mg), calcium keto-valine (86 mg), calcium hydroxyl-methionine (59 mg), L-lysine monoacetate (105 mg), L-threonine (53 mg), L-histidine (38 mg), and L-tyrosine (30 mg). Inclusion of amino acids through oral supplements as an additional source of nitrogen resulted in a mean total protein prescription (from food and supplements) of 0.35 g/kg per day in the VLpD group. The content of cholesterol in the VLpD and LpD diets was 60–80 and 90–130 mg/day, respectively. The daily salt amount was almost 2 g higher in LpD than VLpD (i.e., in the diet containing 2300 Kcal, which was the most frequently prescribed, the whole sodium content was 1260 mg/day [53 mEq=3.1 g NaCl] and 540 mg/day [22 mEq=1.3 g NaCl], respectively, in LpD and VLpD diets) (14,15).

At the first visit, all patients were recommended to minimize added salt to keep daily sodium and phosphorus intake (16,17). As an additional difference, the two diets contained a different percentage of vegetable proteins that was equal to 48% in LpD and 66% in VLpD (14,15).

Patients prepared their food at home and were instructed not to modify the food intake during the study.

Table 1 shows the composition of LpD and VLpD. Regarding phosphorus intake, LpD contained 600–700 mg/day, and VLpD contained 350–420 mg/day (lower than 42% versus LpD) according to the tables for food composition of the Italian National Research Institute for Food and Feeding (Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione in Italia; http://www.inran.it/646/tabelle_di_composizione_degli_alimenti.html). The calcium content was 1189–1324 and 1078–1349 mg/day in LpD and VLpD, respectively ($P$=0.86) (Table 1).

Investigators were instructed to keep the therapy in use before starting the study period unchanged, including angiotensin converting enzyme (ACE) inhibitors, sartanics, statins, and fish oil. Phosphorus, calcium, and PTH levels were treated according to the Kidney Disease Outcomes Quality Initiative guidelines; phosphate binders were not administered during the study period. Noteworthy, 20% of patients were assuming paricalcitol.

FGF23 concentrations were measured in each patient using the intact human FGF23 ELISA (Kainos Laboratories, Tokyo, Japan) that uses two monoclonal antibodies to epitopes on either side of the cleavage site and recognizes the intact molecule. The Kainos Intact FGF23 assay is standardized to measure FGF23 in picograms per milliliter; it has a lower limit of detection of 3 pg/ml and intraassay and interassay coefficients of variation of less than 5%. The FGF23 assays were performed using stored plasma or serum according to the specified kit direction (18–20). All samples were measured in duplicate after a single thaw, with the mean value used in subsequent analysis. The blood was drawn in the morning, and the patients were fasting.

Laboratory chemistries were planned at randomization and the end of each study period. Assays included urea, creatinine, sodium, potassium, phosphate, calcium, intact PTH, hemoglobin, albumin, C-reactive protein, bicarbonate, and FGF23. Samples for urea nitrogen, phosphate, sodium, and creatinine were obtained from 24-hour urinary collections. The tubular reabsorption of phosphate, fractional excretion of $P$, and serum phosphate were used as measures of renal phosphate handling (21). Sodium fractional excretion and n-protein catabolic rate were also evaluated.

### Statistical Analyses

Statistical analyses were carried out using the SPSS statistics package version 18.0. Data were described as mean ± SD. Comparisons were made using Student $t$ test, Mann–Whitney $U$ test, and one-way ANOVA as appropriate. Regression analysis for repeated measures and multivariate analysis were performed using binary logistic regression to determine independent predictors of FGF23 increase. A $P$ value $<$ 0.05 was considered significant.

### Results

Table 2 shows clinical characteristics of patients at baseline. Patients (21 males and 11 females) had a mean age of
hypertensive agents, 18 patients assumed three agents, 66 years and a mean body weight of 77 kg; 90% of patients used ACE inhibitors, 65% used sartanics, 45% used β-blockers, and 33% used other drugs. All patients used diuretics (50±500 mg/day). Five patients assumed four anti-hypertensive agents, 18 patients assumed three agents, and 9 patients assumed two agents; 19 (59%) patients used statins, 24 (75%) patients used fish oil, and 10 (31%) patients used both drugs.

Table 3 shows clinical and laboratory data at the end of each study period (patients ingesting VLpD diet were compared with themselves ingesting LpD). After only 1 week, VLpD reduced the following parameters: diastolic BP (P<0.002), serum urea (P<0.004), urinary urea (P<0.001), n-protein catabolic rate (P<0.001), sodium serum (P<0.001), urinary sodium (P<0.03), sodium fractional excretion (P<0.001), serum P (P<0.001), urinary P (P<0.008), and bicarbonate (P<0.03); it increased tubular reabsorption of phosphate (P<0.04) (Table 3).

Mean FGF23 levels were 167.8±44.1 pg/ml during LpD and 111.6±8.5 pg/ml during VLpD (P<0.001), with a reduction of 33.5% (Figure 1). We also observed a reduction of 12% of serum P levels and 34% of urinary P. Fractional excretion of P was lower during VLpD as a consequence of a reduced P intake (obviously, we observed an increase of tubular reabsorption of phosphorus). FGF23 levels reduction correlated with P levels reduction (for LpD: y=3.62x+0.11, r=0.31; for VLpD: y=2.93x+0.12, r=0.42) (Figure 2) and correlated with phosphaturia (for LpD: y=628.7x+0.54, r=0.22; for VLpD: y=149.3x+1.56, r=0.48) (Figure 3).

Table 4 shows the multivariate analysis model; serum P (odds ratio=1.11, confidence interval=1.04–1.19) and urinary P (estimates of the P intake; odds ratio=1.22, confidence interval=1.12–1.37) were significant determinants of high FGF23 levels. The increased protein intake (observed during LpD compared with VLpD) was the major determinant of FGF23 levels in CKD patients (odds ratio=1.85, confidence interval=1.51–2.23).

Discussion
In the present study, we found that a reduction of protein and phosphate intake with VLpD supplemented with keto-analогues and essential amino acids, without using phosphate binders, lowered FGF23 in CKD patients not yet on dialysis. It has been shown that an increase of FGF23 develops in the early stages of CKD (22). The work by Ix et al. (23) showed that mild decrements of GFR or albuminuria were independently associated with higher FGF23 in 792 patients with stable CV disease and normal kidney function to moderate CKD. The work by Gutierrez et al. (24) showed that FGF23 levels increased in 80 CKD patients before the development of serum mineral abnormalities and were independently associated with serum phosphate and calcitriol deficiency. The work by Isakova et al. (25) studied 3879 CKD patients (stages 2–4) from the Chronic Renal Insufficiency Cohort study. They showed that FGF23 levels increased significantly with decreasing GFR and that high FGF23 levels were more common than OHPT and hyperphosphatemia in all strata of GFR (25).

FGF23 controls phosphate and vitamin D metabolism, and it is a primary regulator of renal phosphate excretion (10,26). It is inversely associated with GFR (9); this mechanism is fundamental to maintain serum phosphate at constant levels as renal function worsens. As a consequence of the physiologic action of FGF23, early CKD is characterized by high levels of FGF23 with normal serum P levels. Such an adaptation may have deleterious trade-offs, because
high FGF23 is associated with a high risk of death in ESRD patients, independent of serum phosphate (27). An association between FGF23 and left ventricular hypertrophy (28,29) has recently been reported in CKD patients and elderly people in the general population (30). Furthermore, FGF23 can be an intermediary in the development of sHPT, because it inhibits the activity of 1α-hydroxylase. These findings highlight the potential role of FGF23 as a sensitive and early biomarker of phosphorus disorders in CKD. Therefore, it is of paramount importance to begin early monitoring of P levels, sHPT, and FGF23 levels. Some studies have already shown that dietary phosphorus restriction and the concurrent use of phosphate binders lowered urinary phosphate excretion, with a rapid decrease in phosphorus absorption and FGF23 levels (31–33). Isakova et al. (25) randomized 16 normophosphataemic CKD stages 3 and 4 patients to lanthanum carbonate or placebo, and they ingested a diet containing 750 or 1500 mg dietary phosphorus. They showed that dietary P restriction and lanthanum carbonate reduced urinary P excretion without changes in serum P and FGF23 (25). In contrast, the group assigned to a higher dietary P and placebo had a significant increase in FGF23 levels (32).

The work by Oliveira et al. (31) compared calcium acetate with sevelamer hydrochloride, and the latter was more effective at lowering FGF23 levels with reduction of urinary phosphate and without changing phosphate levels. The work by Shigematsu et al. (33) compared calcium-containing phosphate binders and lanthanum carbonate in 36 hemodialysis hyperphosphatemic patients and showed a reduction of phosphate and FGF23 levels. Recently, Yilmaz et al. (34) observed a reduction of serum phosphate levels and FGF23 in 100 hyperphosphatemic CKD-4 patients with phosphate binders.

Our study is the first to evidence that a conspicuous protein restriction (0.3 g/kg body wt per day), with essential amino acids and ketoanalogues and without the administration of phosphate binders, is able to reduce FGF23 in CKD patients not yet on dialysis (stages 3 and 4) compared with a usual LpD (0.6 g/kg per day). Healthy

| Table 3. | Hematological differences in LpD and VLpD steps (data are as shown as mean ± SD unless otherwise indicated) |
|-----------------|-----------------|-----------------|
| Systolic BP, mmHg | 130±23 | 126±27 |
| Diastolic BP, mmHg | 77±8 | 71±7 |
| Azotemia, mg/dl | 106±47 | 74±37 |
| Creatinine, mg/dl | 2.8±0.8 | 3.0±0.7 |
| Na, mmol/L | 143±2 | 139±3 |
| K, mmol/L | 5.0±0.5 | 5.2±0.7 |
| Calcium, mg/dl | 9.3±0.6 | 9.1±0.6 |
| Phosphorus, mg/dl | 4.0±0.5 | 3.5±0.6 |
| Hemoglobin | 11.7±1.7 | 12.0±0.9 |
| Albumin, g/dl | 4.3±0.2 | 4.2±0.4 |
| PTH, pg/ml | 202±77 | 181±74 |
| Reactive C-protein, mg/L | 3.4±2.6 | 3.2±2.7 |
| Bicarbonate, mEq/L | 24±3 | 22±4 |
| FGF23, pg/ml | 167.8±44.1 | 111.6±8.5 |
| Diuresis, ml/d | 2492±677 | 2271±414 |
| Fosfaturia, mg/d | 703±276 | 464±209 |
| RTP, percent | 63.7±11.9 | 70.0±12.4 |
| Sodium, mmol/d | 178±92 | 137±43 |
| UUN, mg/d | 10.4±1.2 | 4.8±0.9 |
| FeNa, percent | 2.94±1.48 | 1.49±1.39 |
| n-Protein catabolic rate, g/kg body wt | 0.73±0.07 | 0.34±0.09 |
| Creatininuria, mg/d | 1.04±0.27 | 1.07±0.36 |
| Creatinine clearance, ml/min | 29.3±8.2 | 29.1±8.9 |
| FeP, percent | 37.3±5.4 | 30.0±6.1 |
| LpD, low-protein diet; VLpD, very-low-protein diet; PTH, parathyroid hormone; FGF23, fibroblast growth factor 23; RTP, tubular reabsorption of phosphorus; UUN, urinary urea nitrogen; FeNa, fractional excretion of sodium; FeP, fractional excretion of phosphorus.

Figure 1. | Single FGF23 levels during the low-protein diet (LpD) and the very low-protein diet (VLpD; P<0.0001).
subjects have a P intake of about 1200 mg (31,35); the diets used in our study contained 350–420 (VLpD) and 600–700 (LpD) mg/d of phosphorus, with a reduced P intake of 68% and 34%, respectively, compared with healthy subjects. In physiologic conditions, intestinal absorption of P is about 80% of ingested P (29,36), and urinary excretion is about 62% of ingested P; 400 mg are lost with feces. In our study, urinary excretion of P was about 700 mg in LpD (suggesting 87% intestinal P absorption) and 460 mg in VLpD (suggesting 92% intestinal P absorption). It is plausible that, during both diets, patients ingested a greater amount of P with food containing additives that increased P dietary intake (37). Additionally, beverages other than water contain a P amount that may affect dietary P intake; in fact, the work by Savica et al. (35) showed that beer contains 254 mg/100 ml, Coca-Cola contains 277 mg/100 ml, and red wine contains 848 mg/100 ml. It is possible to reach a P intake of 688 mg/100 ml with red wine and beer and 1125 mg/100 ml with red wine and Coca-Cola. Nevertheless, in our study, patients showed a reduction of urinary P about 40% greater with VLpD compared with LpD, and the intense and fast reduction of P intake obtained with only 1 week of VLpD was sufficient to reduce FGF23 levels, which is contrary to what has been showed in healthy subjects (12,38–41) and CKD patients using phosphate binders (29,31).

Figure 2. | FGF23 and P levels correlation. □, VLpD (y=2.93x+0.12, r=0.42, P<0.01); ◆, LpD (y=3.62x+0.11, r=0.31, P<0.05). The dashed line is the correlation between FGF23 and serum phosphate in VLpD, whereas the thick line is the correlation between FGF23 and serum phosphate in LpD.

Figure 3. | FGF23 and urinary P levels correlation (y=8.52x+484, r=0.35, P<0.05). □, VLpD (y=149.3x+1.56, r=0.48, P<0.01); ◆, LpD (y=628.7x+0.54, r=0.22, P=0.23). The dashed line is the correlation between FGF23 and phosphaturia in VLpD, whereas the thick line is the correlation between FGF23 and phosphaturia in LpD.
Moreover, the vegetarian diet allows a minor absorption of dietary phosphate because of a reduced bioavailability of phosphate contained in vegetables compared with meat and a reduced intestinal absorption, which was recently showed in the work by Moe et al. (42) in a crossover trial of nine patients with moderate CKD. Already in 1984 and in agreement with our data, the work by Portale (41) showed, in eight children aged 6–17 years with moderate renal insufficiency (GFR=45–4 ml/min), that dietary phosphate intake reduced serum phosphate of 0.2 mg/dl and urinary phosphate of 12 mg/kg per day; it increased 1,25OH2D of 16 pg/ml (41). Our results agree with the recent work by Gutierrez et al. (43) that evaluated 1261 participants of the Health Professionals Follow-Up Study. They showed that, in subjects with largely preserved kidney function, a higher phosphate intake was associated with higher FGF23 values (43).

Numerous recent evidences confirmed this observation, giving strength to the updated trade-off hypothesis (44) that introduces the increase of FGF23 in subjects with mild and severe CKD as a determinant of hyperparathyroidism. The present study shows that a very-low-protein intake leads to an important reduction of dietary P, P levels, and P renal filtration; these effects of the VLpD on phosphate determine a reduction of FGF23 levels without using phosphate binders. Our data disagree with the work by Oliveira et al. (31), because we show that VLpD reduces serum and urinary phosphate. Also, it is evident that dietary control of P intake is certainly the first phase of therapeutic control of hyperphosphatemia in CKD and the most physiologic tool, and it also has a stronger effect on dietary phosphate intake reduction compared with phosphate binders.

If FGF23 is directly correlated to CV and coronary events (29,45–47), vascular alterations (48), left ventricular hypertrophy (28,30), and mortality (9,49,50), it seems obvious that a VLpD may be useful to prevent CV events reducing FGF23 levels.

Our study has some limitations. The sample size was small, but we used a crossover design to increase the power of the study. The study patients were enrolled in only one center, where the VLpD and LpD are widely used. Finally, the duration of the study was short, and we did not evaluate the effects of the two diets and FGF23 lowering on hard outcomes.

In conclusion, this study confirm that the only use of a VLpD is to reduce oral phosphorus intake, and consequently, it is able to reduce FGF23 levels more than an LpD without using phosphate binders. Additional studies are needed to confirm our data. The identification of FGF23 as an early biomarker of mineral and bone disorders may change the therapy for phosphorus metabolism in early CKD and patients with normal levels of phosphorus that are, at the moment, not treated according to the current guidelines (51).

Disclosures
None.

References
17. Benini O, D’Alessandro C, Gianfaldoni D, Cupisti A: Extra-phosphate load from food additives in commonly eaten foods: A

### Table 4. Multivariate analysis model showing significant predictors of higher FGF23 in patients with CKD (corrected for creatinine clearance and phosphorus intake)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
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<td>Phosphorus (0.1 mg/dl increase)</td>
<td>1.11</td>
<td>1.04–1.19</td>
<td>0.03</td>
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<tr>
<td>Phosphaturia (10 mg/d increase)</td>
<td>1.22</td>
<td>1.12–1.37</td>
<td>0.02</td>
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<tr>
<td>LpD (yes versus no)</td>
<td>1.85</td>
<td>1.51–2.23</td>
<td>0.005</td>
</tr>
<tr>
<td>FGF23; OR, odds ratio; CI, confidence interval; LpD, low-protein diet.</td>
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**Received**: July 27, 2011 **Accepted**: January 20, 2012

Published online ahead of print. Publication date available at www.cjasn.org.
Correction
Di Iorio B, Di Micco L, Torraca S, Sirico ML, Russo L, Pota A, Mirenghi F, Russo D: Acute Effects of Very-Low-Protein Diet on FGF23 Levels: A Randomized Study. Clin J Am Soc Nephrol 7: 581–587, 2012; published ahead of print February 23, 2012, doi:10.2215/CJN.07640711. The authors would like to report an error in their manuscript. On page 585, line 2, we wrote (using reference 35 as the reference) that beer contains 254 mg/100 ml, Coca-Cola contains 277 mg/100 ml, and red wine contains 848 mg/100 ml of phosphorus. The correct concentrations are as follows: beer, 110 mg/L; cola beverages, 171 mg/L; and red wine, 303 mg/L. We apologize to the readers for the mistake.