



## Changes in Fibroblast Growth Factor 23 and Soluble Klotho Levels After Hemodialysis Initiation

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**Rationale & Objective:** Patients with chronic kidney failure have markedly elevated fibroblast growth factor 23 (FGF-23) levels and decreased soluble Klotho levels. However, no studies have examined the effects of hemodialysis initiation on the levels of these hormones and other parameters of mineral metabolism.

**Study Design:** Prospective single-arm study.

**Setting & Participants:** 20 individuals with incident kidney failure initiating hemodialysis.

**Exposure:** Initiation of hemodialysis. Dose adjustments of phosphate binders and vitamin D receptor activators and use of calcimimetics, erythropoiesis-stimulating agents, and intravenous iron were prohibited.

**Outcomes:** Changes in serum levels of FGF-23, soluble Klotho, and other biochemical parameters of mineral metabolism, measured before and after each hemodialysis session, for a total of 4 sessions over 5 days.

**Analytical Approach:** Repeated-measures analysis of variance.

**Results:** At baseline, participants had 18-fold higher median FGF-23 levels and 1.6-fold lower mean soluble Klotho levels compared with age- and sex-matched healthy individuals. Initiation of hemodialysis led to progressive reductions in serum phosphorus, intact parathyroid hormone, and FGF-23 levels, with dialysis-related fluctuations. No reductions were observed in levels of  $\alpha_1$ -microglobulin, which has molecular weight comparable to FGF-23. The magnitude of the FGF-23 level reductions was strongly associated with concomitant changes in serum phosphorus levels but not with the changes in intact parathyroid hormone levels. Soluble Klotho levels did not change after the initiation of hemodialysis.

**Limitations:** Single-arm design, small sample size, short follow-up period.

**Conclusions:** Initiation of hemodialysis in patients with chronic kidney failure led to progressive reductions in FGF-23 levels in association with reductions in serum phosphorus levels. These results suggest that phosphorus is a strong inducer of FGF-23 production and that regulation of FGF-23 production is a rapid process.

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Fibroblast growth factor 23 (FGF-23) is a bone-derived endocrine hormone that regulates phosphate and vitamin D metabolism. FGF-23 primarily acts on the kidneys to induce urinary phosphate excretion and suppress 1,25-dihydroxyvitamin D ( $1,25[\text{OH}]_2\text{D}$ ) synthesis.<sup>1-3</sup> The physiologic functions of FGF-23 are mediated by binding to FGF receptors and the single-pass transmembrane protein Klotho,<sup>4</sup> which form a specific receptor complex for FGF-23.<sup>5,6</sup> The secretion of FGF-23 by osteocytes is known to be regulated by phosphorus,<sup>7</sup> calcium,<sup>8</sup>  $1,25(\text{OH})_2\text{D}$ ,<sup>9</sup> and parathyroid hormone (PTH),<sup>10,11</sup> but the exact mechanism and time scale of the regulation in humans are not completely understood.

In addition to its role as a coreceptor for FGF-23, Klotho is cleaved and acts as a circulating factor called soluble Klotho.<sup>12</sup> Several experimental studies suggest that soluble Klotho may exert multiple pleiotropic effects as a humoral factor.<sup>13-16</sup> Furthermore, a recent study demonstrated that soluble Klotho, like membrane-bound Klotho, can act as a coreceptor to support FGF-23 signaling.<sup>17</sup> The kidney is the principal site of soluble Klotho production<sup>4,18</sup> and its clearance,<sup>19</sup> but the regulation of these processes is largely unknown.

The FGF-23–Klotho system plays a key role in disordered mineral metabolism in chronic kidney disease

(CKD).<sup>20</sup> Circulating FGF-23 levels increase during the progression of CKD,<sup>21,22</sup> presumably as a compensatory response to maintain a normal phosphate balance. However, in patients who progress to chronic kidney failure, the capacity of the kidney to excrete urinary phosphate in response to FGF-23 is progressively declined, finally leading to the development of overt hyperphosphatemia. This process is accompanied by a progressive reduction in  $1,25(\text{OH})_2\text{D}$  levels, which further stimulates PTH secretion.<sup>23</sup> Patients with chronic kidney failure thus commonly manifest hyperphosphatemia, decreased  $1,25(\text{OH})_2\text{D}$  levels, and secondary hyperparathyroidism.

In patients with chronic kidney failure, initiation of hemodialysis has a profound impact on systemic homeostasis, including symptoms of uremia and fluid overload. However, few studies have examined the effect of hemodialysis initiation on mineral metabolism,<sup>24</sup> and no studies to date have addressed the effect on the FGF-23–Klotho system. Owing to the expectation that the initiation of hemodialysis drastically alters the disturbance in mineral metabolism associated with chronic kidney failure, a prospective investigation of changes in FGF-23 and soluble Klotho levels following hemodialysis initiation may provide a clue regarding the still poorly understood regulation

of these molecules. We therefore prospectively examined the effect of hemodialysis initiation on indexes of mineral metabolism and the FGF-23–Klotho system in patients with chronic kidney failure requiring kidney replacement therapy.

## METHODS

### Study Population

Patients were eligible for inclusion if they were 20 years or older, had chronic kidney failure requiring kidney replacement therapy, and were scheduled for the initiation of hemodialysis. Patients were excluded if they switched from peritoneal dialysis to hemodialysis or initiated hemodialysis as combination therapy with peritoneal dialysis.

This study was conducted in accordance with the principles of the Declaration of Helsinki, and all patients provided written informed consent. The study protocol was approved by the Institutional Review Board of Tokai University School of Medicine (16R-047). This study was registered with the UMIN Clinical Trials Registry (UMIN000023536).

### Study Design and Hemodialysis Procedure

This was a prospective single-arm study conducted from July through December 2017. All patients were followed up over 5 days and 4 hemodialysis sessions. To prevent dialysis disequilibrium syndrome, therapy was initiated with 2 hours of dialysis at a low blood flow rate with a small surface area dialyzer. Patients received hemodialysis daily for the first 3 days (days 0, 1, and 2), and on day 4, treatment time, blood flow, and dialyzer size were incrementally increased. A detailed description of the hemodialysis regimen is provided in Table 1. We used dialysate containing 2.75 mEq/L of calcium. During the study period, prescriptions of phosphate binders and vitamin D receptor activators (VDRAs) were unchanged, and administration of calcimimetics, erythropoiesis-stimulating agents (ESAs), or intravenous iron was prohibited.

To compare FGF-23 and soluble Klotho levels in patients with chronic kidney failure with those in healthy individuals, we recruited healthy age- and sex-matched controls from prospective kidney donors. Blood samples were collected before kidney donation and were used for the measurements.

### Biochemical Measurements

We measured the biochemical parameters of mineral metabolism before and after each dialysis session for a total of 8 times. Serum FGF-23 levels were determined using a chemiluminescence immunoassay (Kyowa Medex Co Ltd) that exclusively detects the full-length FGF-23 peptide. Serum soluble Klotho levels were determined using a sandwich enzyme-linked immunosorbent assay kit (Immuno-Biological Laboratories Co, Ltd) that detects circulating soluble Klotho using 2 monoclonal antibodies that specifically recognize the extracellular domain of Klotho.<sup>25</sup> Serum intact PTH levels were determined using an electrochemiluminescence immunoassay (Elecsys PTH; F. Hoffmann-La Roche Ltd). Serum 1,25(OH)<sub>2</sub>D levels were determined using a radioimmunoassay (Immunodiagnosics Systems). Serum  $\alpha_1$ -microglobulin (A1M) was determined using a latex agglutination turbidimetric immunoassay (LZ Test Eiken  $\alpha_1$ -M; Eiken Chemical Co, Ltd). Hemoglobin, serum albumin, creatinine, calcium, phosphorus, alkaline phosphatase, iron, total iron-binding capacity, and ferritin were measured using standard methods. The measured serum calcium levels were corrected for albumin concentration using Payne's formula.<sup>26</sup> Estimated glomerular filtration rate was calculated using the equation best tailored to the Japanese population.<sup>27</sup>

### Statistical Analyses

Data are reported as mean  $\pm$  standard deviation, mean with 95% confidence interval (CI), median and interquartile range (IQR), or percentage. For comparison of characteristics between patients with chronic kidney failure and healthy controls, we used  $\chi^2$  tests, t tests, or Wilcoxon rank sum tests. Associations between parameters were examined using Pearson or Spearman tests. Changes in biochemical parameters were analyzed using repeated-measures analysis of variance followed by Bonferroni post hoc test. Because serum alkaline phosphatase, intact PTH, FGF-23, and ferritin levels were not normally distributed, values were log-transformed for the analysis of variance. Univariate linear regression analysis was used to assess associations between percent change in FGF-23 levels from baseline to day 4 (predialysis) and that of each biochemical parameter. Because we measured 1,25(OH)<sub>2</sub>D at baseline and on day 4 (predialysis) only, we used paired t test for comparison.  $P < 0.05$  was considered statistically significant. All analyses were performed using IBM SPSS Statistics 24 (IBM).

**Table 1.** Hemodialysis Regimen

Day (HD session)	Treatment Time	Dialyzer <sup>a</sup>	Surface Area	Clearance, mL/min				Blood Flow Rate	Dialysate Flow Rate
				Urea	Creatinine	Phosphorus	Vitamin B <sub>12</sub>		
Day 0 (1st HD)	2 h	VPS-13HA	1.3 m <sup>2</sup>	194	187	177	129	120 mL/min	500 mL/min
Day 1 (2nd HD)	2 h	VPS-13HA	1.3 m <sup>2</sup>	194	187	177	129	120 mL/min	500 mL/min
Day 2 (3rd HD)	3 h	VPS-15HA	1.5 m <sup>2</sup>	196	190	182	138	150 mL/min	500 mL/min
Day 4 (4th HD)	4 h	VPS-18HA	1.8 m <sup>2</sup>	197	193	187	148	150 mL/min	500 mL/min

Abbreviation: HD, hemodialysis.

<sup>a</sup>All dialyzers were manufactured by Asahi Kasei Medical Co Ltd.

## RESULTS

## Study Population and Baseline Results

A total of 20 patients with chronic kidney failure and 20 healthy age- and sex-matched controls were included in the study. Baseline characteristics of the study population are summarized in Table 2. According to the inclusion criteria, all patients had chronic kidney failure requiring kidney replacement therapy; mean estimated glomerular filtration rate was  $6.0 \pm 1.8$  mL/min/1.73 m<sup>2</sup>. Mean serum calcium level was  $8.7 \pm 0.6$  mg/dL, mean serum phosphorus level was  $5.6 \pm 1.9$  mg/dL, and median intact PTH level was 299 (IQR, 182-356) pg/mL. Median FGF-23 level was 517 (IQR, 300-919) pg/mL, which was markedly higher than that of age- and sex-matched healthy individuals (median, 29 [IQR, 26-34] pg/mL;  $P < 0.001$ ). FGF-23 levels correlated with serum phosphorus levels ( $r = 0.67$  [95% CI, 0.27-0.87];  $P = 0.001$ ) but not with age, kidney function, or serum calcium, intact PTH, or 1,25(OH)<sub>2</sub>D levels. No significant difference in FGF-23 levels was observed between patients receiving and not receiving VDRA. Mean soluble Klotho level was  $297 \pm 107$  pg/mL, which was significantly lower than that of age- and sex-matched healthy individuals ( $464 \pm 180$  pg/mL;  $P = 0.001$ ). Soluble Klotho levels correlated with age ( $r = -0.62$  [95% CI,  $-0.83$  to  $-0.25$ ];  $P = 0.003$ ), but not with kidney function or serum calcium, phosphorus, intact PTH, 1,25(OH)<sub>2</sub>D, or FGF-23 levels. Patients treated with VDRA showed higher soluble Klotho levels than those not treated ( $413 \pm 133$  and  $268 \pm 80$  pg/mL, respectively;  $P = 0.01$ ).

## Changes in Mineral Metabolism After Hemodialysis Initiation

All participants underwent 4 sessions of hemodialysis over 5 days, and there were no missed follow-ups and no withdrawals from the study. Biochemical parameters after hemodialysis are shown in Figure 1. Serum creatinine levels decreased with a postdialysis rebound for each session ( $P < 0.001$ ). Serum calcium levels increased slightly after initiation of hemodialysis ( $P < 0.001$ ), presumably by influx of ionized calcium from the dialysate. In contrast, serum phosphorus levels decreased markedly and progressively with a postdialysis rebound for each session ( $P < 0.001$ ), indicating sufficient removal of phosphorus from the body by hemodialysis. Serum intact PTH levels decreased progressively, particularly at the end of the hemodialysis session ( $P < 0.001$ ; Fig 1D). Serum alkaline phosphatase levels did not change significantly during the study ( $P = 0.3$ ; Fig S1A). There was a nonsignificant tendency toward a decrease in transferrin saturation ( $P = 0.09$ ), but no changes in serum ferritin levels were observed ( $P = 0.4$ ; Fig S1B and C).

## Effect of Hemodialysis Initiation on FGF-23 and Soluble Klotho

After initiation of hemodialysis, FGF-23 levels decreased significantly almost in a linear fashion ( $P = 0.002$ ; Fig 2A).

Table 2. Baseline Characteristics of the Study Population

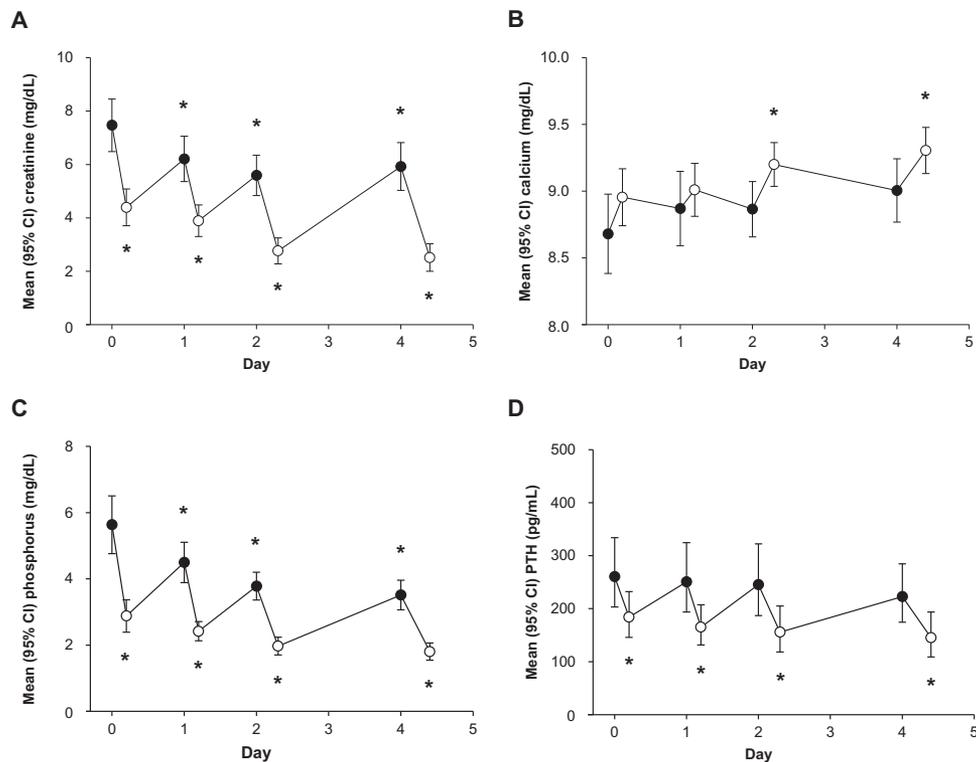
Characteristic	Kidney Failure Patients	Healthy Individuals
No. of patients	20	20
Age, y	69 ± 14	67 ± 4
Male sex	10 (50%)	10 (50%)
Primary cause of kidney failure		
Glomerulonephritis	5 (25%)	—
Diabetes	5 (25%)	—
Hypertension	6 (30%)	—
Others	4 (20%)	—
BMI, kg/m <sup>2</sup>	22.7 ± 3.9	23.3 ± 2.6
Systolic blood pressure, mm Hg	148 ± 22	137 ± 15
Diastolic blood pressure, mm Hg	75 ± 17	78 ± 10
Laboratory tests		
Hemoglobin, g/dL	9.2 ± 1.8	14.0 ± 1.1
Albumin, g/dL	3.2 ± 0.5	4.3 ± 0.2
Creatinine, mg/dL	7.47 ± 2.10	0.75 ± 0.14
Estimated GFR, mL/min/1.73 m <sup>2</sup>	6.0 ± 1.8	71.1 ± 14.6
Calcium, mg/dL	8.7 ± 0.6	9.5 ± 0.4
Phosphorus, mg/dL	5.6 ± 1.9	3.4 ± 0.5
Intact PTH, pg/mL	299 [182-356]	44 [38-53]
ALP, U/L	225 [164-310]	243 [197-271]
1,25(OH) <sub>2</sub> D, pg/mL	10.8 ± 4.9	63.0 ± 11.2
FGF-23, pg/mL	517 [300-919]	29 [26-34]
Soluble Klotho, pg/mL	297 ± 107	464 ± 180
TSAT, %	29 ± 16	—
Ferritin, ng/mL	115 [39-210]	—
Medication		
Calcium carbonate	5 (25%)	—
Lanthanum carbonate	1 (5%)	—
VDRA	5 (25%)	—
ESA <sup>a</sup>	13 (65%)	—

Note: Data are shown as mean ± standard deviation, median [interquartile range], or number (percentage). Conversion factors for units: creatinine in mg/dL to μmol/L, ×88.4; calcium in mg/dL to mmol/L, ×0.2495; phosphorus in mg/dL to mmol/L, ×0.3229.

Abbreviations: 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; ALP, alkaline phosphatase; BMI, body mass index; ESA, erythropoiesis-stimulating agent; FGF-23, fibroblast growth factor 23; GFR, glomerular filtration rate; PTH, parathyroid hormone; TSAT, transferrin saturation; VDRA, vitamin D receptor activator.

<sup>a</sup>Use before the initiation of hemodialysis.

Notably, after the second hemodialysis session, FGF-23 levels continued to decline even in the interdialytic period, suggesting sustained suppression of FGF-23 production by osteocytes. We also measured serum levels of A1M, which has a molecular weight comparable to that of FGF-23 (30 vs 32 kDa, respectively). There was a slightly increasing trend in serum A1M levels ( $P = 0.003$ ) but with no significant difference from baseline at any time point by post hoc tests, suggesting that molecules around this size could not be removed by hemodialysis (Fig 2C). Soluble Klotho levels did not change after initiation of hemodialysis ( $P = 0.1$ ; Fig 2B).



**Figure 1.** Changes in (A) serum creatinine, (B) calcium, (C) phosphorus, and (D) intact parathyroid hormone (PTH) levels after initiation of hemodialysis. Closed circles indicate predialysis values, and open circles indicate postdialysis values. Data for intact PTH were analyzed on the log scale and back-transformed for presentation. Abbreviation: CI, confidence interval. \* $P < 0.05$  versus baseline.

To determine factors responsible for the reductions in FGF-23 levels, we examined associations of percent changes in FGF-23 levels from baseline to day 4 (predialysis) with the biochemical response to hemodialysis. Of the variables examined, only percent changes in serum phosphorus levels were identified as a significant factor associated with percent changes in FGF-23 levels (Table 3). Use of ESAs was not allowed during the study period, but 13 patients (65%) received long-acting ESAs before hemodialysis initiation. These patients showed lower reductions in FGF-23 levels than patients with no prior ESA use ( $-23\% \pm 31\%$  and  $-59\% \pm 12\%$ , respectively;  $P = 0.01$ ). Reductions in FGF-23 levels were comparable between patients treated and not treated with VDRAs ( $-47\% \pm 18\%$  and  $-33\% \pm 33\%$ , respectively;  $P = 0.4$ ) and between patients treated and not treated with calcium carbonate ( $-23\% \pm 50\%$  and  $-40\% \pm 22\%$ , respectively;  $P = 0.5$ ).

### Changes in 1,25(OH)<sub>2</sub>D Levels After Hemodialysis Initiation

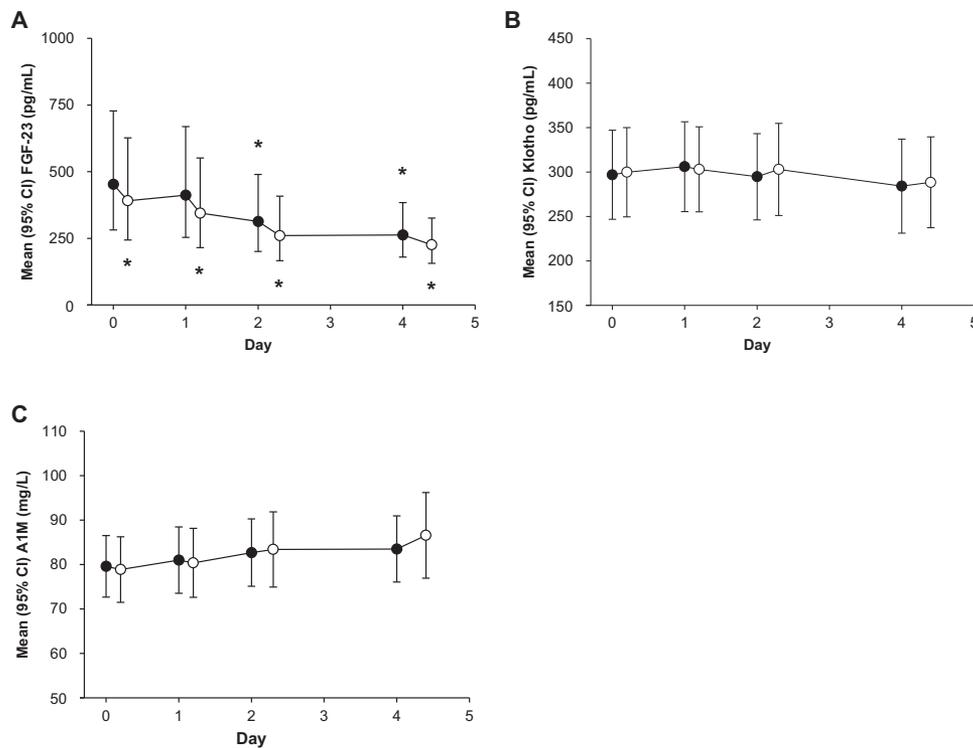
Because we found a marked reduction in FGF-23 levels after the initiation of hemodialysis and FGF-23 is a potent regulator of vitamin D metabolism,<sup>1-3</sup> we next explored the effect of hemodialysis initiation on 1,25(OH)<sub>2</sub>D levels. There was a nonsignificant tendency toward an increase in 1,25(OH)<sub>2</sub>D levels from  $10.8 \pm 4.9$  pg/mL at baseline to

$11.9 \pm 5.5$  pg/mL on day 4 (predialysis;  $P = 0.08$ ); the difference became statistically significant when evaluating percent change from baseline ( $14\% \pm 26\%$ ;  $P = 0.03$ ).

## DISCUSSION

In this prospective study of patients with chronic kidney failure, initiation of hemodialysis led to progressive reductions in FGF-23 levels, together with significant reductions in serum phosphorus and intact PTH levels. The magnitude of the FGF-23 level reductions was strongly associated with concomitant changes in serum phosphorus levels rather than changes in intact PTH levels. FGF-23 levels continued to decline even in the interdialytic period, suggesting sustained suppression of FGF-23 production by osteocytes. Accompanying these changes, there was a slight tendency toward increased 1,25(OH)<sub>2</sub>D levels. Soluble Klotho levels did not change after the initiation of hemodialysis despite the drastic changes in mineral metabolism. To our knowledge, this is the first study to investigate longitudinal changes in FGF-23 and soluble Klotho levels in patients who were beginning hemodialysis therapy.

Prior studies have examined whether blood purification therapies could remove FGF-23 in maintenance hemodialysis patients. One study showed that FGF-23 levels were lowered by hemodiafiltration or hemoperfusion but not by



**Figure 2.** Changes in (A) fibroblast growth factor 23 (FGF-23), (B) soluble Klotho, and (C)  $\alpha_1$ -microglobulin (A1M) levels after initiation of hemodialysis. Closed circles indicate predialysis values, and open circles indicate postdialysis values. Data for FGF-23 were analyzed on the log scale and back-transformed for presentation. Abbreviation: CI, confidence interval. \* $P < 0.05$  versus baseline.

**Table 3.** Univariate linear Regression Analyses Between Percent Changes in FGF-23 and Other Biochemical Parameters

Variable	$\beta$ Estimate	SE	$P$	95% CI
Calcium, per 1% change	2.03	1.33	0.1	-0.76 to 4.82
Phosphorus, per 1% change	1.41	0.24	<0.001	0.91 to 1.91
Intact PTH, per 1% change	0.37	0.29	0.2	-0.24 to 0.99
ALP, per 1% change	0.57	0.48	0.3	-0.44 to 1.58
1,25(OH) <sub>2</sub> D, per 1% change	0.46	0.26	0.09	-0.08 to 0.99
Soluble Klotho, per 1% change	0.85	0.49	0.1	-0.18 to 1.89
TSAT, per 1% change	-0.12	0.24	0.6	-0.63 to 0.39
Ferritin, per 1% change	0.05	0.13	0.7	-0.22 to 0.32

Abbreviations: 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; ALP, alkaline phosphatase; PTH, parathyroid hormone; TSAT, transferrin saturation.

hemodialysis.<sup>28</sup> In another study using dialysate containing 3.0 to 3.5 mEq/L of calcium, FGF-23 levels increased after hemodialysis,<sup>29</sup> which could be explained by the increased calcium exposure from dialysate because calcium stimulates FGF-23 production.<sup>8</sup> Thus, results of these

studies indicate that FGF-23 could not be removed by hemodialysis. In the present study, we initiated hemodialysis with a low-dose regimen to prevent dialysis disequilibrium syndrome, so it is further unlikely that FGF-23 was removed by hemodialysis. We confirmed that hemodialysis did not lower serum levels of A1M, which has a molecular weight comparable to that of FGF-23. Therefore, the decreased FGF-23 levels following the initiation of hemodialysis are largely attributable to the suppressed production of FGF-23 by osteocytes.

The physiologic regulation of FGF-23 synthesis by osteocytes is still not completely understood, but phosphorus, calcium, 1,25(OH)<sub>2</sub>D, and PTH are known to be key drivers of FGF-23 production.<sup>7-11</sup> In the current analysis, the degree of FGF-23 reduction was strongly associated with changes in serum phosphorus levels but not with changes in levels of other bone mineral parameters, suggesting that the marked reduction in serum phosphorus levels was the primary contributor to the decreased FGF-23 levels. The implication is that phosphorus drives increased Fgf23 transcription by unknown mechanisms and also decreases Galnt3 expression (encoding N-acetylgalactosaminyl-transferase 3), which is involved in FGF-23 posttranslational cleavage.<sup>30</sup> Our findings are not consistent with prior studies that showed no reduction in postdialysis FGF-23 levels in maintenance

hemodialysis patients,<sup>28,29</sup> but this discrepancy could be explained by the difference in study populations. Although prior studies included patients receiving maintenance hemodialysis who had relatively stable phosphate homeostasis with dialysis-related fluctuations, our study included patients who had progressive phosphate retention with declining kidney function before starting hemodialysis. In this setting, the initiation of hemodialysis resulted in a progressive negative phosphorus balance by removing phosphorus from the body, which could have caused the sustained suppression of FGF-23 production. In previous studies of nondialysis patients with CKD, phosphate binders lowered FGF-23 levels. However, the effects were weak and could be observed only after weeks or months of treatment.<sup>31-33</sup> In contrast, following kidney transplantation, FGF-23 levels decreased by >50% within 1 week, along with the occurrence of posttransplantation hypophosphatemia.<sup>34</sup> Together with results of previous studies, our results suggest that in the presence of marked changes in systemic phosphate balance, osteocytic production of FGF-23 can be changed on a day-to-day basis.

Although phosphorus is likely the most important driver of the reductions in FGF-23 levels after initiation of dialysis, other factors might have also affected the reductions in FGF-23 levels. Because at the time of the study design, there were preliminary data suggesting that erythropoietin induces FGF-23 expression, which were published subsequently,<sup>35,36</sup> the present study prohibited the use of ESAs during the study period. However, quite a few patients received long-acting ESAs before hemodialysis initiation, the effects of which might have persisted during the study. Interestingly, these patients showed lower reductions in FGF-23 levels after initiation of hemodialysis than did the other patients, which might be explained by the persistent effects of the ESAs. We also found reductions in intact PTH levels at the end of each hemodialysis session, which were likely caused by the influx of ionized calcium from the dialysate. Although there was no significant association between changes in FGF-23 and intact PTH levels, we cannot exclude the possibility of involvement of PTH in the regulation of FGF-23 in the long term. There also remains the possibility that improvement of metabolic acidosis and use of iron also contributed to the reductions in FGF-23 levels because these factors are known to affect FGF-23 transcription, production, or cleavage.<sup>37,38</sup>

Another interesting finding of this study was that the low 1,25(OH)<sub>2</sub>D levels tended to increase, albeit slightly, after initiation of hemodialysis. Decreased kidney synthesis of 1,25(OH)<sub>2</sub>D in chronic kidney failure has traditionally been attributed to diminished kidney mass and phosphate retention, but recent studies provide compelling evidence that FGF-23 plays a major role in the decreased 1,25(OH)<sub>2</sub>D synthesis, especially in early to moderate CKD.<sup>20-23</sup> Our findings support this notion and further suggest that even in patients with chronic kidney failure requiring kidney replacement therapy, kidney 1 $\alpha$ -

hydroxylase activity is in part functionally inhibited by FGF-23.

Our results may have implications for interpreting previous studies showing an association of elevated FGF-23 levels with increased mortality. Higher FGF-23 levels have been strongly associated with mortality in nondialysis patients with CKD<sup>39</sup> and incident hemodialysis patients.<sup>40</sup> These observations and subsequent experimental studies<sup>41-43</sup> helped foster the concept that elevated FGF-23 is not just a biomarker of phosphate-mediated toxicity but is directly toxic itself. However, the association between elevated FGF-23 level and mortality has been less pronounced in maintenance hemodialysis patients.<sup>44</sup> In this regard, our findings of decreased FGF-23 levels after initiation of hemodialysis may support the hypothesis that FGF-23 levels during maintenance hemodialysis could less accurately reflect the antecedent burden of the cardiovascular toxicity of FGF-23 and phosphate in the predialysis period, which limits the ability of these parameters to predict mortality. To address this possibility, future studies should target patients with advanced CKD transitioning to dialysis and compare the predictive ability of FGF-23 levels in the predialysis period, at the initiation of hemodialysis, and during maintenance dialysis.

Soluble Klotho is produced by proteolytic cleavage of the extracellular domain of membrane Klotho, which is expressed predominantly in the kidney.<sup>4,18</sup> In line with this, we found significantly lower soluble Klotho levels in patients with chronic kidney failure than in healthy individuals. However, the reductions were <50%, suggesting that soluble Klotho could also be produced by organs other than the kidneys. These findings are in agreement with the previous observation that soluble Klotho level was markedly decreased but not completely absent in mice with targeted deletion of Klotho in the kidneys.<sup>18</sup> Notably, recent studies suggest that parathyroid<sup>45</sup> and pituitary<sup>46</sup> glands are also sites of soluble Klotho production, which might explain the less marked reductions in soluble Klotho levels in patients with chronic kidney failure. In the present study, the initiation of hemodialysis did not change soluble Klotho levels despite the marked changes in mineral metabolism. These observations are consistent with our previous study showing that cinacalcet treatment lowered PTH levels and concomitantly lowered serum calcium, phosphorus, and FGF-23 levels but did not produce large changes in soluble Klotho levels.<sup>47</sup> Taken together, these observations suggest that factors involved in mineral and bone metabolism are not the major regulator of soluble Klotho production.

Strengths of this study include its prospective design, frequent measurements of biochemical parameters, and the study protocol that prohibited dose adjustments of phosphate binders<sup>31-33</sup> and VDRAs<sup>9</sup> and the use of calcimimetics,<sup>48</sup> ESAs,<sup>35,36</sup> and intravenous iron,<sup>49</sup> all of which could affect FGF-23 levels.

There are also several important limitations. First, we did not collect dialysate samples for FGF-23

measurements and thus could not completely exclude the possibility of removal of FGF-23 by hemodialysis. Second, this study lacked a concurrent control group because all study participants had chronic kidney failure requiring kidney replacement therapy. Third, the association between changes in FGF-23 and serum phosphorus levels was strong but should not be interpreted as causal. Fourth, we did not measure C-terminal FGF-23 levels, which might have provided additional information on the relative contribution of increased *Fgf23* transcription and decreased posttranslational cleavage. However, it has been observed that most of the circulating FGF-23 is intact in patients with chronic kidney failure.<sup>50</sup> Fifth, we measured soluble *Klotho* using the commercially available assay alone<sup>25</sup> and did not assess whether similar results could be obtained with an immunoprecipitation-immunoblot assay.<sup>19</sup> Other potential limitations include small sample size, short follow-up period, and restriction to Japanese patients, which may limit the generalizability of our results.

In conclusion, we demonstrated that the initiation of hemodialysis in patients with chronic kidney failure led to progressive reductions in FGF-23 levels in association with reductions in serum phosphorus levels. Our results support the current view that phosphorus is a strong inducer of FGF-23 production by osteocytes and further suggest that regulation of FGF-23 synthesis can occur more rapidly than previously thought. Furthermore, our results support the importance of serum phosphorus management for suppressing FGF-23 production, which may have diverse toxic effects. We also confirmed that soluble *Klotho* levels were decreased in patients with chronic kidney failure, but these levels did not change after the initiation of hemodialysis, suggesting that factors involved in mineral metabolism play a limited or insignificant role in the regulation of soluble *Klotho* production.

## SUPPLEMENTARY MATERIAL

### Supplementary File (PDF)

**Figure S1:** Changes in TSAT (A), ferritin (B), and ALP (C) levels after initiation of hemodialysis.

## ARTICLE INFORMATION

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