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FGF-23 is Elevated by Chronic Hyperphosphatemia

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ABSTRACT The identification and characterization of FGF-23 has provided an opportunity to gain new insight into phosphorus metabolism. Circulating FGF-23 promotes renal excretion of phosphorus, and FGF-23 is measurable in the serum of normal subjects. Serum levels of FGF-23 are elevated in patients with renal phosphate wasting disorders such as tumor induced osteomalacia, X-linked hypophosphatemia and fibrous dysplasia. However, the factors that alter its serum concentration are not known. The study of serum FGF-23 is confounded by the fact that high serum calcium, PTH, and any other putative phosphotonins, have similar effects on serum and urine phosphorus. To circumvent the confounding effect of serum PTH and calcium, we studied serum FGF-23 and phosphate levels in patients with chronic hypoparathyroidism and hyperphosphatemia. Serum was collected in the morning after an overnight fast from three groups: 1) 9 patients with chronic hypoparathyroidism on stable treatment with calcium and calcitriol, 2) 9 patients with primary hyperparathyroidism, and 3) 77 normal controls. Patients with hypoparathyroidism had predictably higher levels of serum phosphorus than patients with hyperparathyroidism or normal controls (5.6± 1.1, 3.1± 0.6, and 3.1± 0.5 mg/dL, mean ± 1 SD, respectively ($p<0.01$ for hypoparathyroid vs: either group)). They also had higher levels of FGF-23 (150±120 vs: 70±60, or 55±20 RIU/ml, respectively ($p<0.05$ vs: either group)). In conclusion, serum FGF-23 levels are elevated in patients with hyperphosphatemia and chronic hypoparathyroidism, suggesting a feedback system in which serum FGF-23 responds to serum phosphorus and regulates it. However, in the setting of chronic hypoparathyroidism, the degree of elevation of FGF-23 is insufficient to normalize serum phosphorus.

Introduction

FGF-23 was first identified as the gene product mutated in patients with autosomal dominant hypophosphatemic rickets (ADHR)(1). Subsequently, it was shown that FGF-23 is markedly over expressed in tumors that cause renal phosphate wasting and impaired vitamin D metabolism(2, 3). FGF23 concentrations are elevated in serum from patients with tumor induced osteomalacia (TIO) and X-linked hypophosphatemic rickets (XLH)(4, 5). In addition to TIO and XLH, FGF-23 has been shown to be elevated in patients with fibrous dysplasia of bone (FD). In FD, the magnitude of elevation of serum FGF-23 correlates with the degree of phosphate wasting and the amount of the skeleton affected with FD, and it appears that the source of FGF-23 in FD is affected osteogenic cells (6). Additionally, nude mice implanted with FGF-23-expressing CHO cells exhibit renal phosphate wasting (7), and direct injection of FGF-23 into mice results in renal phosphate wasting (2). Transgenic mice over expressing FGF-23 demonstrate serum biochemical and skeletal abnormalities similar to those seen in patients with XLH and TIO(8, 9), and mice with deletion of FGF-23 show hyperphosphatemia (10). All of these data establish that excessive serum levels of FGF-23 increase renal excretion of phosphorus.

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Regulation of serum FGF-23 in humans has not been extensively studied. FGF-23 has been shown to be present in the serum of normal subjects (6), without age- or gender-related differences. Serum FGF-23 did not respond to variation in serum phosphate levels in normal volunteers, but is elevated in patients with renal failure (11).

The study of FGF-23 in normal physiology may be confounded by the fact that PTH and FGF-23 have some overlapping, and potentially counter-regulating physiologic effects. Both inhibit proximal tubule phosphorus reabsorption, resulting in the lowering of serum phosphorus. Efforts to manipulate serum phosphorus levels in order to study serum FGF-23 responses might cause serum PTH levels to change in a counter regulatory fashion, thus confounding the results. To determine serum FGF-23 levels in patients with hyperphosphatemia and to avoid the potentially confounding effects of PTH and serum calcium, we studied the serum FGF-23 levels in eucalcemic patients with chronic hypoparathyroidism on stable treatment with calcium and calcitriol.

Patients and Methods

Subjects: All subjects gave written informed consent, and the studies were approved by the institutional

Table 1

Diagnosis	n	Male/Female	Age	Median Age	Serum Calcium[a]	PTH[b]
Normal Control	77	38/39	21-83	48	2.25 ± 0.10	ND
Hyperparathyroid	9	4/5	22-68	46	2.52 ± 0.17	114 ± 91.1
Hypoparathyroid	9	3/6	7-75	31	2.15 ± 0.15	4.6 ± 3.5

[a] normal range: 2.05-2.50 mmol/L, [b]normal range: 6.0–40.0 pg/ml), ND = not done

review boards of the National Institutes of Health and Indiana University-Purdue University Indianapolis/Clarian. Nine consecutive patients with hypoparathyroidism, 9 consecutive patients with hyperparathyroidism, and 77 normal volunteers were studied. Demographics of the subjects are shown in Table1. Hypoparathyroidism was diagnosed on the basis of hypocalcaemia with very low or undetectable serum levels of parathyroid hormone. The etiology of hypoparathyroidism was post-surgical (5 patients), idiopathic (3 patients), and autoimmune (1 patient). All hypoparathyroid patients were on stable doses of calcitriol and calcium to yield serum calcium levels near the lower limit of normal. The median and range of the daily dose of calcitriol in the hypoparathyroid group were 1.0, and 0.5 – 2.5 μg/day, respectively, divided, bid. Hyperparathyroidism was diagnosed on the basis of hypercalcemia with concomitant high serum parathyroid hormone levels.

Methods: Serum and urine creatinine, calcium, and phosphorus were measured with commercially available assays. Serum PTH was measured using the Nichols Bionitact assay (Nichols Institute, San Clemente, CA). Serum FGF-23 was measured using a commercially available two-site immunoassay for the FGF-23 C-terminus (Immutopics, San Clemente, CA). All serum samples were collected in the morning after an overnight fast.

Statistical Analyses: Differences between groups were assessed by ANOVA. All means are presented ± 1 SD. When data were not normally distributed, the Tukey-Kramer multiple comparisons post test was used for comparing the differences between the means of the groups. For testing the significance of correlation coefficients in groups that were nonparametrically distributed, the Spearman rho was calculated. These analyses were performed using InStat version 3.0 (GraphPad Software, Inc., San Diego, CA, USA).

Results

As expected, serum phosphorus was significantly higher in patients with hypoparathyroidism than in patients with hyperparathyroidism or control subjects (5.6± 1.1 vs: 3.1± 0.6, or 3.1± 0.5 mg/dL, respectively $p<0.001$) (Table. 2). Serum FGF-23 was higher in patients with hypoparathyroidism than in patients with hyperparathyroidism or control subjects (150±120 vs: 70±60, or 55±20 RIU/ml, respectively $p<0.05$), but serum FGF-23 levels in patients with hyperparathyroidism were not different from those in the normal control group (Table 2, Fig. 1). Individual data points for all patients and the mean ± 1SD for each group are shown in Fig. 1. Serum FGF-23 levels were positively correlated with serum phosphorus levels when all three groups were combined (Spearman rho = 0.46, $p < 0.001$), and in the normal control group (Spearman rho = 0.37, $p < 0.001$), but not in either the hypoparathyroid or the hyperthyroid group alone. There was not a significant correlation between serum FGF-23 and either the total daily dose of calcitriol, or the AM serum levels of 1,25-$(OH)_2$-vitamin-D3 in the hypoparathyroid group. There was not a significant correlation between serum calcium and FGF-23 for all groups, nor was there a significant correlation between serum PTH and FGF-23 in the hyperparathyroid group.

Table 2.

Serum Phosphorus (mg/dL)			
Hypoparathyroid (n=9)	5.6 ± 1.1	p<0.001	p<0.001
Hyperparathyroid (n=9)	3.1 ± 0.6	p=NS	
Normal (n=77)	3.1 ± 0.5		
Serum FGF-23 (RU/ml)			
Hypoparathyroid (n=9)	150 ± 120	p<0.05	p<0.01
Hyperparathyroid (n=9)	70 ± 60	p=NS	
Normal (n=77)	55 ± 20		

Values indicate mean ± 1 SD. Differences between groups were calculated by ANOVA using the Tukey-Kramer multiple comparisons posttest. (the SI unit conversion factor serum phosphorus to mmol/L is 0.32)

Discussion

The study of patients with chronic hypoparathyroidism provides the opportunity to observe changes in serum FGF-23 levels independent of potentially confounding PTH effects. Likewise, in patients with hypoparathyroidism, increasing serum calcium acts directly to decrease serum phosphorus(12). Therefore, to eliminate the effect of changes in the level of serum calcium and PTH, we

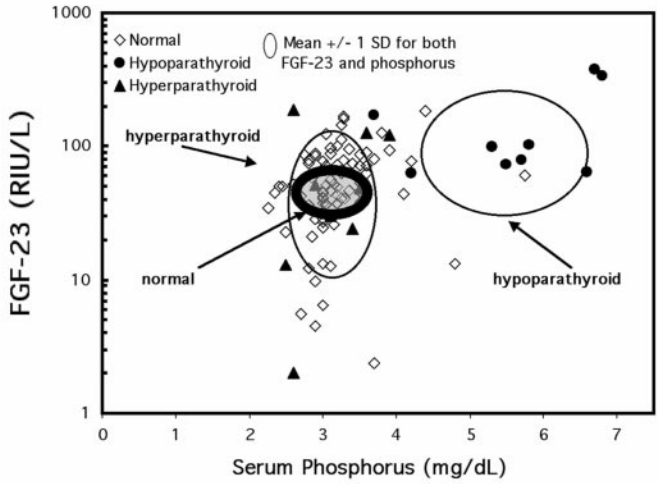

Figure 1. Serum phosphorus and FGF-23. The serum phosphorus and FGF-23 levels for the 3 groups are shown as indicated. The limits of the long and short axes of the ovals are determined by the mean ± 1 SD for both serum phosphorus and FGF-23 for each group as indicated. When compared to the hyperparathyroid and normal control groups, the distinction of the hypoparathyroid group is evident. (*the SI unit conversion factor serum phosphorus to mmol/L is 0.32)

studied patients with hypoparathyroidism who were eucalcemic and on stable treatment for their past hypocalcemia.

Serum FGF-23 levels were elevated in stably treated patients with hyperphosphatemia due to hypoparathyroidism. This would be consistent with a role for serum phosphorus as a regulator of FGF-23 in serum. This suggests a feed back loop wherein, as serum phosphorus rises, FGF-23 increases to reduce serum phosphorus through its phosphaturic properties. However, the data suggest that in the absence of sufficient levels of parathyroid hormone, FGF-23 cannot restore euphosphatemia. This is consistent with what was recently shown in rodents. Hyperphosphatemic parathyroidectomized rats were treated with a dose of FGF-23 that caused hypophosphatemia in intact animals. In aparathyroid animals, this dose reduced serum phosphorus, but did not return it to normal.(13).

The fact that serum FGF-23 was not higher in the hyperparathyroid group (in which the mean serum phosphorus was the same as in the normal control group) supports the idea that serum phosphorus, and not PTH, may be a more important regulator of serum FGF-23. While the n is small, the lack of regulation by PTH is supported by the fact that there was not a correlation between serum PTH and FGF-23. While neither the daily dose of calcitriol, nor the AM serum 1,25-$(OH)_2$-vitamin-D3 levels in the hypoparathyroid group correlated with serum FGF-23, it remains possible that serum 1,25-$(OH)_2$-vitamin-D3 may be a regulator of serum FGF-23. Regulation of serum FGF-23 by 1,25-$(OH)_2$-vitamin-D3 has been demonstrated in mice(13).

In pathologic states of renal phosphate wasting and hypophosphatemia (XLH, TIO, FD)(4-6), serum FGF-23 is elevated. In fibrous dysplasia, the only state in which a correlation between serum FGF-23 and phosphorus has been studied, serum FGF-23 is negatively correlated with serum phosphorus(6). Yet, in the groups studied here, serum FGF-23 levels positively correlated with serum phosphorus. This suggests that the higher serum FGF-23 levels shown here in hyperphosphatemic hypoparathyroid patients are a (secondary) physiologic response. An analogy can be made with primary and secondary hyperparathyroidism. The high levels of FGF-23 in patients with TIO, XLH, or fibrous dysplasia are analogous to the pathologically elevated levels of PTH seen in primary hyperparathyroidism, and the higher levels of FGF-23 seen here are secondary and physiologic, as are the elevated levels of PTH in secondary hyperparathyroidism.

In summary, we have shown that FGF-23 is elevated in chronically hypoparathyroid patients with hyperphosphatemia. However, these higher levels of FG-23 do not normalize serum phosphorus concentrations in the setting of calcitriol-treated hypoparathyroidism. Furthermore, the higher levels of FGF-23 in hyperphosphatemic patients and the positive correlation between FGF-23 and serum phosphorus provide evidence that FGF-23 levels are directly or indirectly increased by higher serum phosphorus.

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