Increased circulating levels of FGF23: an adaptive response in primary hyperparathyroidism?

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Abstract

Introduction: Fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH) are major players in the bone–parathyroid–kidney axis controlling phosphate homeostasis. In patients with primary hyperparathyroidism (PHPT), data on the relationship between PTH and FGF23 are scarce and not always concordant.

Objective: The aim of our study was to evaluate the relationship between PTH and FGF23 in patients with PHPT and in euvathroid patients cured after successful parathyroidectomy (PTx).

Patients and methods: Twenty-one patients with PHPT and 24 patients in long-term cure after successful PTx (EuPTH) were studied. All patients underwent biochemical evaluation of renal function, parathyroid status, vitamin D status, bone turnover markers, and serum intact FGF23 levels.

Results: Mean serum FGF23 concentration was significantly higher in PHPT than in EuPTH patients (50.8 ± 6.1 vs 33.1 ± 2.6 pg/ml, P = 0.01). FGF23 levels significantly correlated with PTH levels (r = 0.361, P = 0.02), also after correction for 1,25(OH) 2D levels (r = 0.419, P = 0.01). FGF23 levels showed a significant negative correlation with 1,25(OH) 2D, which was more pronounced in PHPT than in EuPTH patients (r = −0.674, P = 0.001, vs r = −0.509, P = 0.01).

Conclusion: Our findings suggest that in PHPT, FGF23 levels are increased independent of 1,25(OH) 2D levels. The more pronounced negative relationship between FGF23 and 1,25(OH) 2D in the presence of high circulating PTH levels suggests that the increase in FGF23 levels may be an adaptive mechanism to counteract the PTH-induced increase in 1,25(OH) 2D levels, although not completely overriding it.

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Introduction

Parathyroid hormone (PTH) and the active metabolite of vitamin D (1,25(OH) 2D) are prime regulators of calcium homeostasis but also have significant effects on phosphate homeostasis by downregulating or upregulating the sodium phosphate co-transporters in the proximal tubules of the kidneys and in enterocytes of the intestinal tract (1–8). However, the major player of the bone–kidney axis controlling phosphate homeostasis has been shown to be fibroblast growth factor 23 (FGF23). FGF23 acts as a phosphaturic factor by the same mechanism of action as PTH, downregulating the co-transporters NaPi2a and NaPi2c in the kidney after binding to its receptor, FGFR1, in the presence of Klotho (9–11). FGF23 also decreases 1,25(OH) 2D synthesis in the proximal tubules by direct inhibition of the 1α-hydroxylase enzyme (9, 10, 12).

FGF23 is predominantly produced and secreted by osteocytes in bone (9, 10). This process is positively regulated by 1,25(OH) 2D, via a vitamin D response element in the fgf23 promoter (9, 13–15). The process is also regulated by serum phosphate, although the exact mechanism by which this is achieved remains unclear. Extracellular phosphate does not appear to directly stimulate FGF23 mRNA levels or fgf23 promoter activity in osteoblastic cultures (9, 14). Data on the effect of changes in phosphate intake on FGF23 concentrations are inconsistent, with different responses observed with short-term or long-term alterations in phosphate intake (16–20). It has also been shown that early and rapid changes in renal phosphate excretion occur following a high-phosphorus meal, independent of FGF23, PTH, secreted frizzled-related protein (sFRP4), or 1,25(OH) 2D, suggesting the presence of an intestinal ‘phosphate sensor’, although its exact biochemical nature is not known (21–25).

The PTH/PTHrP receptor (PTHR1) is present on osteocytes (26) and constitutive activation of this receptor has been shown to upregulate FGF23 mRNA expression in vitro (27, 28). Administration of PTH (1–34) in mice and in healthy individuals is associated with an increase in 1,25(OH) 2D and in serum FGF23 levels and with a decrease in serum phosphate levels (13, 28, 29). In contrast, although intermittent administration of PTH to postmenopausal women
with osteoporosis induced an increase in 1,25(OH)₂D
and in FGF23 levels, this was not associated with a
decrease in serum phosphate levels (30). Taken
together, these data suggest that PTH is a regulator of
FGF23 synthesis and that this is likely to be independent
of serum phosphate concentrations.

In patients with primary hyperparathyroidism
(PHPT), data on the relationship between PTH and
FGF23 are scarce and not always concordant.
Compared with healthy controls, circulating FGF23
levels have been found to be elevated in patients
with PHPT before parathyroidectomy (PTx) (31, 32) and
and to decrease immediately post-operatively (32), supporting
the notion that PTH stimulates FGF23 secretion.
However, this post-operative normalization of FGF23
levels was not observed in all studies (31, 33), or was
observed only transiently post-PTx, with FGF23 levels
returning to the originally high pre-operative values
7 days after surgery (32). The latter data suggest a
possible alteration in FGF23 regulation, independent of
PTH levels, in patients with PHPT. The aim of our study
was to address the relationship between PTH and
FGF23 in patients with PHPT and in those with this
disorder after cure following successful PTx.

Patients and methods

Study population

Twenty-one consecutive patients with PHPT, which was
untreated, persistent or recurrent after PTx and
24 consecutive euparathyroid patients who had a
successful PTx for sporadic PHPT at the Leiden
University Medical Center (LUMC) were invited and
agreed to take part in the study over an 18 months
period. All patients were under regular follow-up at the
Outpatient Clinic of the Department of Endocrinology
and Metabolic Diseases of the LUMC, with patients with
persistent hyperparathyroidism being followed more
closely than those cured after PTx, who were mostly
seen at 1- to 2-year intervals.

The diagnosis of PHPT was established on the basis of a
serum PTH concentration above the upper limit of the
normal laboratory reference range (> 8 pmol/l) in the
presence of a high or inappropriately normal serum
calcium concentration (> 2.55 mmol/l). Eight of
these latter patients had PTH concentrations of
13.6 ± 2.2 pmol/l (range 8.4–27.4 pmol/l) in the
presence of a normal serum calcium (serum calcium
2.46 ± 0.02, range 2.38–2.52 mmol/l) and in the
absence of vitamin D deficiency (25(OH)D₃ 55.6 ± 7.2,
range 35–93 nmol/l). Four of these eight patients had a
genetically confirmed MEN1 mutation, the other four
patients had evidence for a parathyroid adenoma on
localization studies and became hypercalcemic under
vitamin D supplementation.

A diagnosis of cure was based on sustained normal
serum calcium and PTH concentrations more than 6
months after PTx.

All patients and controls had to have a creatinine
clearance > 60 ml/min to be included in the study to
preclude the confounding effect of renal impairment on
FGF23 levels. All patients and controls had a 25(OH)
vitamin D₃ level of > 30 nmol/l except for five patients
who had levels between 25 and 28 nmol/l. These five
patients were, however, hypercalcemic (2.72 ± 0.02,
range 2.67–2.80 mmol/l) with increased PTH levels
(sodium PTH 23.5 ± 9.0, range 8.6–54.3 pmol/l) and
high-normal 1,25(OH)₂D₂ levels (serum 1,25(OH)₂D₂
142 ± 20, range 87–205 pmol/l), which was the reason
to withhold the vitamin D supplementation.

Methods

Serum biochemistry Serum concentrations of calcium
(reference range 2.15–2.55 mmol/l), albumin (reference
range 34–48 g/l), phosphate (reference range 0.90–1.50 mmol/l), and creatinine (reference range
44–80 μmol/l) were measured using semi-automated
techniques. Creatinine clearance was calculated using
the Modification of Diet in Renal Disease (MDRD)
formula. Serum alkaline phosphatase (ALP; reference
range 40–120 U/l) was measured using a fully auto-
minated P800 modulator system (Roche BV). Serum
P1NP (a marker of bone formation) and β-CTX (a
marker of bone resorption) were determined using the
E-170 system (Roche BV). Serum concentrations of
intact PTH (reference range 1.5–8 pmol/l) were
measured using the Immulite 2500 (Siemens Diagnos-
tics, Breda, Holland). Serum 25-hydroxycholecalciferol
(25(OH)D₃; reference range 30–120 nmol/l) was
measured using the LIAISON 25-OH Vitamin D TOTAL
assay (DiaSorin S.A./N.V., Bruxelles, Belgium) and
1,25(OH)₂ vitamin D was measured using LIAISON
1,25-OH₂ Vitamin D TOTAL assay (DiaSorin S.A./N.V.).
Serum intact FGF23 (reference range 18–50 pg/ml (34))
was measured using an immunometric assay (Kainos
Laboratories, Inc., Tokyo, Japan; intra-assay coefficient
of variation (CV) 6% and inter-assay CV 10%).

Statistical analysis

Statistical analysis was performed using the SPSS 16.0
software (SPSS, Inc., Chicago, IL, USA). Results
are expressed as mean ± S.E.M. unless otherwise stated.
χ² test and Student’s t-test were used as appropriate
for categorical variables and continuous variables. Pearson
correlation coefficients were calculated to assess
correlations between FGF23, PTH, 1,25(OH)₂D, creati-
nine clearance, phosphate, and calcium. Serum PTH,
FGF23, and 1,25(OH)₂D levels are shown in Table 1 in
absolute values, but were log transformed before
statistical correlation and regression analysis to correct
for skewness. The relationship between several biochemical variables and FGF23 was investigated by backward regression analysis. A probability level of random difference of $P < 0.05$ was considered significant.

The study was approved by the local ethics committee and informed consent was obtained from all patients prior to inclusion in the study.

## Results

Patients with PHPT did not differ significantly in age, gender, weight, body mass index (BMI), and renal function from those in long-term cure after successful PTx (EuPTH; Table 1).

Mean serum calcium and PTH concentrations were significantly higher and mean serum phosphate and $25(OH)$ vitamin D$_3$ concentrations were significantly lower in the PHPT group compared with the EuPTH group. However, serum $1,25(OH)_2$D concentrations and the bone turnover markers, ALP, P1NP, and CTX, were significantly increased in the PHPT group compared with the EuPTH group (Table 1).

Mean serum FGF23 concentration was significantly higher in patients with PHPT than in EuPTH patients (50.8 ± 6.1 vs 33.1 ± 2.6 pg/ml, $P = 0.01$; Table 1). There was a significant positive relationship between PTH and FGF23 levels when PHPT and EuPTH patients were analyzed together ($r = 0.361$, $P = 0.02$; Fig. 1), and this relationship was sustained and more pronounced after correction for $1,25(OH)_2$D levels ($r = 0.419$, $P = 0.01$). There was no significant relationship between PTH and FGF23 when PHPT and EuPTH patients were analyzed separately ($r = 0.187$, $P = 0.4$, vs $r = 0.114$, $P = 0.6$ respectively).

There was also no significant relationship between PTH and $1,25(OH)_2$D levels in either PHPT patients ($r = -0.269$, $P = 0.3$) or EuPTH patients ($r = 0.016$, $P = 0.9$) or when both groups were analyzed together ($r = 0.061$, $P = 0.7$).

In patients with PHPT, there was a significant negative correlation between FGF23 and $1,25(OH)_2$D levels ($r = -0.674$, $P = 0.001$; Fig. 2). This relationship remained significant, albeit less marked, in EuPTH patients ($r = -0.509$, $P = 0.01$; Fig. 2). The negative relationship between FGF23 and $1,25(OH)_2$D remained significant when all patients were pooled together ($r = -0.393$, $P < 0.01$). Using backward stepwise
regression analysis, we also demonstrate that FGF23 levels exhibit significant and independent associations with PTH and 1,25(OH)2D levels ($\beta = 0.372, P = 0.015$, and $\beta = -0.429, P = 0.003$ respectively; Table 2).

There was no significant relationship between FGF23 concentrations and creatinine clearance or serum phosphate concentrations in either PHPT patients ($r = 0.085, P = 0.7$, and $r = 0.349, P = 0.09$ respectively) or EuPTH patients ($r = -0.398, P = 0.06$ and $r = -0.247, P = 0.3$ respectively). Also using backward stepwise regression analysis, creatinine clearance and serum phosphate levels failed to emerge as significant modulating factors for FGF23 levels in this model ($\beta = -0.033, P = 0.811$ and $\beta = -0.068, P = 0.642$ respectively; Table 2).

There was also no significant relationship between FGF23 levels and all three markers of bone turnover, serum ALP activity, P1NP, or CTX concentrations, in either PHPT or EuPTH patients after correction for PTH and 1,25(OH)2D levels.

**Discussion**

Data from our study show that patients with PHPT have higher levels of FGF23 than cured controls and that this increase is independent of 1,25(OH)2D levels. We further demonstrate a significant negative relationship between FGF23 and 1,25(OH)2D levels, that is more pronounced in patients with PHPT suggesting that FGF23 at least partially antagonizes the stimulatory effects of PTH on the 1α-hydroxylase enzyme, although not totally overriding it.

Data on FGF23 levels in PHPT and in the euparathyroid state following successful PTx are scarce and not always concordant. Two studies (31, 33) demonstrated no significant difference in pre- and post-PTx FGF23 levels, but a further study (32) showed a return of FGF23 levels to high pre-operative levels several days after PTx. The authors of this latter paper (32) suggested that one of the reasons for these discrepant results may be the post-operative use of active vitamin D metabolites or analogues in their patients, which had not been taken into consideration in the interpretation of their results. To our knowledge, FGF23 levels have never been previously evaluated in long-term euparathyroid patients after successful PTx. Our findings from this study suggest that the increase in FGF23 levels observed in PHPT is reversible when the euparathyroid state is achieved by cure after successful PTx, providing that renal function is not impaired.

Although the cross-sectional design of our study does not allow the definitive determination of a causal relationship between PTH and FGF23, our data is in keeping with recently published data in parathyroidectomized rats, in which a direct relationship between PTH and FGF23 independent of 1,25(OH)2D is demonstrated in the presence of high but not low levels of PTH (35).

In the presence of high PTH and FGF23 levels in patients with PHPT, it is intriguing that a significant number of these patients do not develop hypophosphatemia despite chronic exposure to the two phosphahtic hormones, PTH and FGF23. In keeping with previous observations (13, 30), indeed only 11 of our 22 patients with PHPT (50%) had phosphate levels below the lower limit of normal (<0.90 mmol/l). This suggests that in PHPT, factors other than PTH, FGF23, or their combined effect may play a role in phosphate homeostasis. A clear contender is 1,25(OH)2D. The net effect of 1,25(OH)2D on gut, kidney, bone, and parathyroids is to increase serum phosphate levels, by upregulating NaPi2b co-transporter expression in the intestinal tract (1, 7) and NaPi2 co-transporter (NaPi3) gene in the kidney (3, 8) and by directly reducing PTH synthesis and secretion by the parathyroid (29).

**Table 2** Result of multiple regression analysis, demonstrating a significant association between FGF23, PTH, and 1,25(OH)2D.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>B</th>
<th>S.E.M.</th>
<th>$\beta$</th>
<th>t</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH</td>
<td>0.895</td>
<td>0.353</td>
<td>0.372</td>
<td>2.534</td>
<td>0.015</td>
</tr>
<tr>
<td>1,25(OH)2D</td>
<td>-0.192</td>
<td>0.061</td>
<td>-0.429</td>
<td>-3.124</td>
<td>0.003</td>
</tr>
<tr>
<td>Phosphate</td>
<td>-7.959</td>
<td>16.996</td>
<td>-0.068</td>
<td>-0.468</td>
<td>0.642</td>
</tr>
<tr>
<td>MDRD</td>
<td>-0.039</td>
<td>0.160</td>
<td>-0.033</td>
<td>-0.241</td>
<td>0.811</td>
</tr>
</tbody>
</table>
In our study, patients with PHPT had significantly increased 1,25(OH)2D levels compared with eurathyroid patients, but also demonstrated significantly increased FGF23 levels. A new hypothesis has been recently proposed to explain the need for two phosphaturic hormones, PTH and FGF23, with the former repressed and the latter induced by 1,25(OH)2D (36). The suggested negative feedback loop includes FGF23-induced inhibition of 1,25(OH)2D synthesis. It has been proposed that these counter-regulatory effects of FGF23 on the bone–kidney axis have the physiological task of securing the maintenance of serum phosphate levels, thus providing protection against the hyperphosphatemia-related soft tissue and vascular calcifications (37–40). A possible explanation for the antagonizing effect of FGF23 on the 1α-hydroxylase enzyme may be the shorter half-life of PTH compared with the longer half-life of FGF23 (41).

Our findings from this study extend our insight into the role of FGF23 in pathological states by showing that in PHPT, FGF23 production is increased in the presence of high circulating PTH levels and that this increase is reversible after the eurathyroid state is achieved following successful PTx. The more pronounced negative relationship between FGF23 and 1,25(OH)2 vitamin D in patients with PHPT suggests that in these patients the increase in FGF23 levels may be an adaptive mechanism to counteract the PTH-induced increase in 1,25(OH)2D levels, although not completely overriding it.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

18. Larsson T, Nisbeth U, Ljunggren O, Juppner H & Juhlin L. Circulating concentration of FGF-23 increases as renal function declines in patients with chronic kidney disease, but does not


