(1-34) Parathyroid Hormone Infusion Acutely Lowers Fibroblast Growth Factor 23 Concentrations in Adult Volunteers

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Summary

Background and objectives Fibroblast growth factor 23 (FGF23) regulates phosphorus and vitamin D metabolism. Parathyroid hormone (PTH) infusion for 24 hours stimulated FGF23 secretion in healthy volunteers. The extent to which this was due to a direct stimulatory effect of PTH versus an indirect effect of increasing 1,25-dihydroxyvitamin D [1,25(OH)2D] levels was unclear.

Design, setting, participants, & measurements Changes in FGF23 in 26 adults undergoing 6-hour (1-34) PTH infusion were examined, focusing particularly on the effects of PTH on FGF23 in the early period of infusion before sustained increases in 1,25(OH)2D.

Results FGF23 levels declined in parallel with serum phosphate during infusion (P<0.05 for both), with both analytes decreasing within the first hour and reaching their respective nadirs at 6 hours. These changes were observed despite no change in 1,25(OH)2D levels during the first hour and a significant increase in 1,25(OH)2D from baseline after 6 hours (P<0.001). There were no differences in these responses by race. However, modest racial differences in the phosphaturic response to (1-34) PTH were observed (P=0.04 for interaction), with a higher rate of increase in fractional phosphate excretion in blacks than in whites.

Conclusions During short-term (1-34) PTH infusion, FGF23 levels decreased in parallel with serum phosphate levels and despite significant increases in 1,25(OH)2D. When coupled with the results of prior longer-term infusion studies, these findings suggest that acute increases in PTH initially act to suppress FGF23 secretion, perhaps to mitigate urinary phosphate losses, before the stimulatory effect of 1,25(OH)2D on FGF23 eventually begins to predominate.

Introduction

Fibroblast growth factor 23 (FGF23) is a central regulator of phosphorus and vitamin D metabolism (1). Early increases in FGF23 secretion play a key role in the initiation and progression of disordered bone and mineral metabolism in CKD (2,3). Studies have also shown that increased FGF23 is independently linked with cardiovascular disease events and mortality in individuals across a broad range of kidney function (4–8), establishing excess FGF23 as a novel risk factor for adverse clinical outcomes. Together, these data have spurred interest in identifying the primary mediators of increased circulating FGF23 levels in CKD and in the general population.

FGF23 synthesis is regulated by both systemic and local bone-derived factors (9). The primary systemic regulators of FGF23 seem to be dietary phosphorus intake and circulating levels of 1,25-dihydroxyvitamin D [1,25(OH)2D] (9). Recent research suggests that parathyroid hormone (PTH) also regulates FGF23 secretion. In rats with experimentally induced kidney failure, parathyroidectomy decreased serum FGF23 levels before, and prevented their rise after, the induction of kidney disease (10). Analogous results were observed in a transgenic mouse model of primary hyperparathyroidism, in which FGF23 levels fell in parallel with decreasing PTH levels after parathyroidectomy (11). In addition, continuous subcutaneous infusion of (1-34) PTH increased circulating FGF23 levels in mice and in parathyroidectomized rats (10,12). Notably, however, other studies showed opposite effects (13–15).

Human studies also suggest that PTH regulates FGF23 secretion. Continuous infusion of (1-34) PTH for 24–46 hours raised serum FGF23 levels in healthy volunteers and in patients with ESRD (16,17). Similarly, daily subcutaneous injections of (1-34) PTH increased FGF23 levels within 3 months of initiating therapy in postmenopausal women with established osteoporosis (18). Collectively, these results indicate that exogenous PTH administration stimulates FGF23 secretion. The reasons for these findings were less clear. Although PTH directly upregulated fgf23 expression in osteoblast-like cells in vitro (10), circulating FGF23 levels invariably increased subsequent to...
sustained increases in 1,25(OH)2D levels \textit{in vivo} \cite{10,16–18}. These results make it difficult to determine the extent to which these latter findings were the result of a direct stimulatory effect of PTH as opposed to an indirect effect of elevated 1,25(OH)2D levels. Given the recent suggestion that PTH-induced increases in FGF23 expression underlie high FGF23 levels in CKD \cite{10}, delineating direct versus indirect effects of PTH on FGF23 synthesis is of interest. Accordingly, we examined acute changes in plasma FGF23 levels in adult volunteers undergoing short-term (1-34) PTH infusion, focusing specifically on the early period of the infusion—just after the initiation and before 1,25(OH)2D levels would normally begin to increase—to distinguish the direct effects of PTH from those of 1,25(OH)2D.

Materials and Methods

Study Participants
Twenty-six adult volunteers from greater Miami-Dade County, Florida, were recruited to participate in a study examining acute changes in renal phosphate handling in response to (1-34) PTH infusion. The primary aims of this study were to investigate potential racial differences in renal sensitivity to the phosphaturic stimulus of PTH, as well as to examine changes in FGF23 in response to acute increases in PTH using a short-term infusion protocol \cite{19,20}. To be eligible, potential participants needed to be either black or white and \(\geq 18\) years of age. Exclusion criteria included the following: known history or laboratory evidence of kidney disease as indicated by an estimated GFR < 60 ml/min per 1.73 m² or an abnormal urinalysis; a current medical condition known to affect phosphorus metabolism, such as parathyroid or thyroid disease; current use of medications known to affect phosphorus metabolism, including oral phosphorus supplements, regular antacid use, and anticonvulsants; or screening laboratory evidence of abnormal phosphate levels (serum phosphate > 4.6 or < 2.5 mg/dL), abnormal calcium levels (serum total calcium > 10.6 or < 8.5 mg/dL), or severe anemia (hemoglobin < 8 g/dL for women and < 9 g/dL for men). Six participants with HIV infection were allowed to participate because they were otherwise healthy (normal blood counts, no history of opportunistic infections), had no evidence of kidney disease on laboratory screening results, were either not taking antiretroviral medications or were taking a stable regimen of these medications for at least 6 months, and were not taking any other medications known to affect phosphorus metabolism. The Human Subjects Research Office at the University of Miami Miller School of Medicine approved this study, and all participants provided written, informed consent.

Study Protocol
After completing a screening visit during which eligibility for study participation was confirmed, study participants provided a 24-hour urine collection for estimation of average daily urinary phosphorus and calcium excretion as well as calculation of creatinine clearance. On the morning of the infusion visit, participants arrived at the University of Miami Clinical Research Center after at least an 8-hour overnight fast. Upon arrival, participants were weighed and had an intravenous catheter placed in the antecubital vein of each arm. One hour after the intravenous catheter was placed, baseline blood and urine samples were collected and the (1-34) PTH infusion was initiated at a rate of 0.055 \(\mu g/\text{kg per hour}\). This rate was based on previous studies examining the effect of (1-34) PTH infusion on renal mineral ion handling in healthy volunteers \cite{21–23}. The time of the initiation of the infusion (8:30 AM) was standardized for all participants. After the infusion was initiated, blood and urine samples were collected every 30 minutes for the first 2 hours, and then every hour after that for the remaining 4 hours. To calculate hourly urine mineral ion excretion, total urine output was recorded each hour starting 1 hour before the initiation of the infusion. Participants remained fasting throughout the 6-hour infusion, but were encouraged to drink at least 100 ml of water per hour to maintain a water diuresis. After the completion of the 6-hour infusion, the catheters were removed and participants were discharged home.

(1-34) PTH for infusion was individually prepared for each participant by adding sufficient commercially available teriparatide (Forteo; Eli Lilly, Indianapolis, IN) to 150 ml of 0.9% normal saline to deliver 0.055 \(\mu g/\text{kg per hour}\) for 6 hours. Before adding teriparatide to the saline solution, 25% heat-deactivated human albumin was added to achieve a final concentration of 5 mg of albumin per milliliter of saline to ensure that teriparatide peptide did not adhere to the wall of the saline bag or infusion tubing.

Laboratory Methods
Measurements of serum and urine phosphate, calcium, and creatinine concentrations were performed at each time point using standard laboratory assays. PTH and FGF23 measurements were performed at each time point in previously unthawed plasma samples stored at \(-80^\circ\text{C}\) at the time of sample collection. PTH was measured using an ELISA that exclusively detects the full 1-84 peptide (Immutopics, San Clemente, CA) with coefficients of variation < 6%. FGF23 was measured using a second-generation ELISA that detects two epitopes in the C-terminal domain of the peptide (Immutopics) with coefficients of variation < 5%. We measured 1,25(OH)2D at baseline, 1 hour, and 6 hours using liquid chromatography tandem mass spectrometry (Quest Diagnostics, San Juan Capistrano, CA). Fractional excretion of phosphate (\(\text{FE}_{\text{PO}_4}\)) and calcium (\(\text{FE}_{\text{Ca}}\)) were calculated using the following formula: \((\text{urine mineral}) \times (\text{serum creatinine}) / (\text{serum mineral}) \times (\text{urine creatinine})\).

Statistical Analyses
Linear mixed-effects models were used to examine changes in serum phosphate, calcium, PTH, FGF23, 1,25(OH)2D, \(\text{FE}_{\text{PO}_4}\), \(\text{FE}_{\text{Ca}}\), and hourly urine mineral ion excretion during the 6-hour infusion. In these models, time represented the repeated-measures factor and individuals were treated as random-effects terms. We first tested whether the rate of change of the outcome variable differed according to race by including interaction terms (race \(\times\) time) in these models. When interaction was detected \((P<0.10)\), we analyzed separate models by race. When no interaction was detected, we examined the main effect of
time. When there was a significant effect of time, we localized individually significant changes in postbaseline time points by comparing them with the baseline level using ordinary least-squares linear regression. In all models, FGF23 was log-transformed to approximate a normal distribution. In addition, the area under the curve for each analyte during the 6-hour infusion was calculated using the linear trapezoidal rule (24). Two-sided \( P < 0.05 \) was considered statistically significant. All analyses were performed using SAS software (version 9.2.1; SAS Institute, Cary, NC).

Results

Participant Characteristics

Table 1 depicts baseline characteristics and fasting laboratory measurements of the study participants. There were no statistically significant differences between black and white participants with respect to other demographic or clinical characteristics or baseline indices of mineral metabolism.

Change in Blood Parameters in Response to (1-34) PTH Infusion

Figure 1 depicts the changes in blood phosphate, FGF23, 1,25(OH)\(_2\)D, total calcium, and (1-84) PTH levels during the 6-hour infusion. There were no statistically significant racial differences in the rate of change of any of these parameters (\( P > 0.10 \) for interaction between race and time), nor any overall differences by race (\( P > 0.05 \) for main effect of race). Therefore, data for black and white participants were pooled to examine the change in these analytes in the overall group over time. Serum phosphate levels modestly decreased from baseline over the course of the 6-hour infusion (Figure 1A) (\( P < 0.001 \) for main effect of time). Similarly, plasma FGF23 levels moderately decreased during the 6 hours (Figure 1B) (\( P = 0.03 \) for main effect of time). The decrease in plasma FGF23 levels seemed to coincide with that of serum phosphate throughout the 6 hours, with both analytes acutely falling within the first hour of infusion and declining to their respective nadirs at the final time point. These changes occurred despite no significant change in 1,25(OH)\(_2\)D levels during the first hour of infusion and a significant increase in 1,25(OH)\(_2\)D levels from baseline after 6 hours of infusion (Figure 1C) (\( P < 0.001 \) for main effect of time). Consistent with prior studies (21–23), serum total calcium levels increased during the infusion (Figure 1D) (\( P < 0.001 \)), whereas plasma (1-84) PTH concentrations gradually declined (Figure 1E) (\( P < 0.001 \)).

Change in Urinary Mineral Ion Excretion in Response to (1-34) PTH Infusion

The change in FEPO\(_4\), over time in response to (1-34) PTH significantly differed by race (\( P = 0.04 \) for interaction between race and time). As depicted in Figure 2A, FEPO\(_4\) increased from baseline within the first 60 minutes of infusion in both black and white participants, with the slope of increase being slightly steeper in blacks. In contrast to FEPO\(_4\), the magnitude and rate of increase in mean urinary phosphate excretion per hour did not differ by race (data not shown).

Figure 2B depicts the concurrent changes in FECa by race. Although the rate of change of FECa did not differ by group (\( P = 0.10 \)), there were significant overall differences by race (\( P = 0.03 \) for main effect of race), with the area under the curve being approximately 50% lower in blacks compared with whites. Furthermore, there were significant within-group differences over time, with FECa decreasing from baseline in both groups after an initial increase during the first hour (\( P < 0.001 \)). Similar racial differences were noted for changes in mean urinary calcium excretion during the 6 hours (data not shown).

Discussion

The primary finding of this study was that plasma FGF23 levels acutely decreased in response to short-term (1-34) PTH infusion in adults. These changes were observed within the first 60 minutes of the infusion; coincided with the concurrent, marked increase in urinary phosphate loss; and occurred despite no change in 1,25(OH)\(_2\)D levels during the first hour of infusion and a significant increase in 1,25(OH)\(_2\)D after 6 hours of infusion. When coupled with longer-term infusion studies showing a delayed rise in FGF23 levels in response to PTH-induced increases in circulating 1,25(OH)\(_2\)D (16,17), the current results suggest that acute increases in PTH initially act to suppress FGF23 secretion, perhaps to mitigate urinary phosphate losses, before the stimulatory effect of elevated 1,25(OH)\(_2\)D levels eventually begins to predominate, leading to an increase in FGF23.

The mechanisms underlying the decrease in FGF23 levels in this study were unclear, but we can speculate concerning several possibilities. It is conceivable that (1-34) PTH directly inhibited FGF23 synthesis. Supporting this possibility is a previous study showing that daily subcutaneous injections of recombinant human (1-34) PTH in mice for 20 days reduced circulating FGF23 levels and decreased fgf23 expression in calvaria collected ex vivo (15). In direct contrast to these findings, however, more recent studies showed that continuous infusion of (1-34) PTH in mice and parathyroidectomized rats had the exact opposite effects (10,12). Other studies showed that (1-34) PTH had no

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Table 1. Study participant characteristics at baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall (N=26)</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>36±11</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>5/26 (19)</td>
</tr>
<tr>
<td>Black, n (%)</td>
<td>10/26 (63)</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>26±4</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>132±44</td>
</tr>
<tr>
<td>Serum phosphate (mg/dl)</td>
<td>3.4±0.5</td>
</tr>
<tr>
<td>24-h urinary phosphate excretion (mg/d)</td>
<td>810±308</td>
</tr>
<tr>
<td>Serum calcium (mg/dl)</td>
<td>9.0±0.3</td>
</tr>
<tr>
<td>24-h urinary calcium excretion (mg/d)</td>
<td>126±569</td>
</tr>
<tr>
<td>(1-84) parathyroid hormone (pg/ml)</td>
<td>29±9</td>
</tr>
<tr>
<td>Fibroblast growth factor 23 (RU/ml)</td>
<td>52 (41, 67)</td>
</tr>
</tbody>
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Results are depicted as mean ± SD, median (interquartile range), or frequencies.
effect on fgf23 expression in bone cells (13,14), raising uncertainty as to whether PTH directly affects FGF23 synthesis at all. Further studies are needed to reconcile these results and to clarify the direct effects (1-34) PTH on FGF23 expression.

FGF23 levels may also have decreased to maintain normal phosphorus balance. Indeed, the greatest decrease in FGF23 levels coincided with the steep increase in urinary FEPO4 during the first 60 minutes of infusion, suggesting a possible renal feedback mechanism whereby FGF23 secretion can be rapidly inhibited in response to marked increases in urinary phosphate loss in order to avoid severe phosphorus depletion. Alternatively, FGF23 levels may have decreased in response to decreasing serum phosphate levels. Arguing against this possibility is a prior study that showed that acute increases in serum phosphate did not alter FGF23 levels in healthy volunteers (25), suggesting that acute changes in serum phosphate do not modulate FGF23 secretion. Future studies utilizing intravenous phosphate infusions to “clamp” serum phosphate levels during PTH infusion are needed to delineate between these possibilities.

The decrease in FGF23 levels in this study is in contrast to the findings of two prior human studies in which FGF23

Figure 1. | Changes in blood biochemical variables in response to 6-hour (1-34) parathyroid hormone infusion. Serum phosphate (A), plasma fibroblast growth factor 23 (B), serum 1,25-dihydroxyvitamin D (C), serum total calcium (D), and plasma (1-84) parathyroid hormone concentrations (E) are presented. Results are reported as mean ± SEM. *P<0.05 (significant differences at individual time points compared with the within-group’s fasting level based on linear regression models).
levels increased after 18–23 hours of (1-34) PTH infusion, subsequent to sustained increases in 1,25(OH)2D. Importantly, the investigators of these prior studies did not measure FGF23 during the first 6 hours of infusion, precluding them from examining whether FGF23 initially declined very early in the infusion, as we observed in this study. There were other noteworthy differences between these studies. Burnett-Bowie et al. measured FGF23 using an assay that exclusively detects the intact peptide, whereas we used an assay that detects both intact peptide and C-terminal fragments. Although this may have contributed to differences in our results, given that Wesseling-Perry et al. used the same assay that we used in this study, variability in FGF23 assays is unlikely to completely explain differences in the effect of PTH on FGF23 between these studies. Similarly, although hourly PTH dose rates varied between these three studies, the dose rate used in this study was comparable with that of Burnett-Bowie et al., making it unlikely to explain observed differences in the direction of the effect of PTH on FGF23. Instead, when the results of these three studies are juxtaposed, they suggest that the effect of acute increases in PTH on FGF23 may consist of two phases. In the first phase, FGF23 secretion is initially suppressed, perhaps to avoid severe phosphorus depletion in the face of PTH-induced increases in urinary phosphate excretion. In addition, given the potent antagonistic effects of FGF23 on 1,25(OH)2D synthesis, an early reduction in FGF23 levels could theoretically play a permissive role in acutely raising 1,25(OH)2D levels, thereby helping PTH fulfill its primary responsibility of defending calcium homeostasis. In the proposed second phase of the response, the stimulatory effect of rising 1,25(OH)2D levels on FGF23 synthesis eventually begins to predominate to avert vitamin D toxicity. If accurate,

Figure 2. Changes in urine variables in response to 6-hour (1-34) parathyroid hormone infusion. Fractional excretion of phosphate (A) and fractional excretion of calcium (B) are presented. □, black participants; ▪, white participants. Results are reported as mean ± SEM. *P<0.05 (significant differences at individual time points compared with the within-group’s fasting level based on linear regression models).
this paradigm would be in keeping with the time-related, biphasic effects of PTH on other parameters of mineral metabolism. For example, studies have shown that PTH has opposing effects on bone turnover and renal cation handling, depending on the frequency, dose, or length of time of PTH administration (26,27).

We previously showed that postprandial \( \text{FEPO}_4 \) was lower in blacks than in whites after a standard breakfast meal (20). Although we found statistically significant racial differences in the rate of \( \text{FEPO}_4 \) increase during (1-34) PTH infusion in this study, these differences were small and would not explain lower \( \text{FEPO}_4 \) in blacks than whites as we previously observed. Future studies must investigate to what extent, if any, these differences contribute to racial variability in phosphorus metabolism.

Because of the short-term period of infusion, circulating PTH levels were not at steady state during the observation period. Thus, the results of this study may not be directly relevant to characterizing the relationships between PTH with FGF23 in the steady state. Nevertheless, these findings offer novel insights into the spectrum of effects that PTH may have on FGF23. In addition, because the infusion was terminated after 6 hours, we were unable to examine the evolution of FGF23 levels over a longer time period. However, previous studies have characterized in detail the temporal changes in FGF23 levels over 24–46 hours of infusion (16,17). Finally, we were unable to examine whether diurnal changes in FGF23 throughout the morning might have affected changes in FGF23 levels during the infusion period. However, given a recent study showing that FGF23 levels, if anything, increase during the morning and early afternoon in healthy volunteers (28), diurnal changes are unlikely to explain the decrease in FGF23 in this study.

Prior studies have shown that FGF23 directly inhibits PTH synthesis and secretion (29,30). When coupled with more recent data showing that PTH directly induces FGF23 synthesis (10), these findings suggest that PTH and FGF23 exist in a classic endocrine feedback loop. The results of this study do not dispute this possibility. Instead, when taken in context of prior studies, these findings suggest that the effects of PTH on FGF23 are not unidirectional, but may vary according to physiologic need. As such, further studies are needed to establish the full spectrum of effects that PTH may have on FGF23 synthesis, especially when trying to decipher what role elevated PTH levels may play in stimulating FGF23 secretion in kidney disease.

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Disclosures
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