CLINICAL STUDY

Fibroblast growth factor-23 is associated with parathyroid hormone and renal function in a population-based cohort of elderly men

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Abstract

Objective: Fibroblast growth factor-23 (FGF23) is a circulating factor involved in phosphate (Pi) and vitamin D metabolism. Serum FGF23 is increased at later stages of chronic kidney disease due to chronic hyperphosphatemia and decreased renal clearance. Recent studies also indicate that FGF23 may directly regulate the expression of parathyroid hormone (PTH) *in vitro*. Therefore, the objective of the current study was to determine the relationship between FGF23, PTH, and other biochemistries *in vivo* in subjects with no history of renal disease.

Design: Serum biochemistries were measured in a subsample of the population-based Swedish part of the MrOS study. In total, 1000 Caucasian men aged 70–80 years were randomly selected from the population.

Methods: Intact FGF23, Pi, calcium, albumin, estimated glomerular filtration rate (eGFR, calculated from cystatin C), PTH, and 25(OH)D₃ were measured. Association studies were performed using linear univariate and multivariate regression analyses.

Results: The median FGF23 level was 36.6 pg/ml, ranging from 0.63 to 957 pg/ml. There was a significant correlation between log FGF23 and eGFR ($r = -0.21$; $P < 0.00001$) and log PTH ($r = 0.13$; $P < 0.001$). These variables remained as independent predictors of FGF23 in multivariate analysis. In addition, log PTH ($\beta = 0.082$; $P < 0.05$) and eGFR ($\beta = -0.090$; $P < 0.05$) were associated with log FGF23 in subjects with eGFR > 60 ml/min. Only eGFR ($\beta = -0.35$; $P < 0.0001$) remained as a predictor of log FGF23 in subjects with eGFR < 60 ml/min.

Conclusions: Serum FGF23 and PTH are associated *in vivo*, supporting recent findings that FGF23 directly regulates PTH expression *in vitro*. Additionally, eGFR is associated with FGF23 in subjects with normal or mildly impaired renal function, indicating that GFR may modulate FGF23 levels independent of serum Pi.

European Journal of Endocrinology 158 125–129

Introduction

Fibroblast growth factor-23 (FGF23) is a circulating phosphaturic factor that plays a critical role in renal phosphate (Pi) reabsorption (1). Numerous activating and inactivating mutations in the human *FGF23* gene have been identified, causing two clinical disorders of disturbed Pi homeostasis: autosomal dominant hypophosphatemic rickets (OMIM#193100) (2) and hyperphosphatemic familial tumoral calcinosis (OMIM#211900) (3, 4).

The interaction between FGF23 and parathyroid hormone (PTH) in physiology as well as in states of disease is not completely understood. Importantly, we recently showed that FGF23 negatively regulates PTH mRNA expression and protein secretion *in vitro* (5). High serum FGF23 in chronic kidney disease (CKD) is a

predictor of secondary hyperparathyroidism (6), although it is unknown whether increased FGF23 is a cause of, or a protective response to, an emerging hyperparathyroidism. In addition, overexpression of FGF23 in transgenic mice causes secondary hyperparathyroidism (7, 8), which could be due to low calcitriol levels and/or hypocalcemia. Finally, serum FGF23 correlates to PTH in pre-dialysis CKD patients (9, 10), and it was also recently shown that FGF23 and PTH were associated in a subgroup of patients with early CKD (10). However, such an association remains to be established in a larger group of subjects with normal or mild impairment of renal function.

The role of FGF23 in CKD has been subject to thorough investigation. Initial reports demonstrated elevated serum FGF23 in CKD, although measurements were performed using an assay detecting both intact, as well as inactive,

C-terminal FGF23 fragments (11–13). Subsequent studies have revealed that intact FGF23 is also increased in CKD, likely as a response to the prevailing hyperphosphatemia (9, 12, 14–16). Notably, increments in FGF23 are usually not detected until other imbalances in mineral ion homeostasis are present (14). In this regard, FGF23 is typically elevated by a 1000-fold or more in subjects with end-stage renal disease (ESRD). Thus, there is a clear association between FGF23 and glomerular filtration rate (GFR) in ESRD (10–12, 14, 16). It is unknown whether FGF23 and GFR are associated in subjects with normal or mildly compromised renal function.

In the current study, we sought to explore the relationship between intact FGF23, PTH, renal function, and other serum biochemistries *in vivo*, employing a large population-based cohort.

Methods

The Swedish MrOS study in Uppsala

The MrOS study is an international multicenter prospective fracture epidemiology investigation, involving elderly men from Hong Kong, USA, and Sweden. The Swedish part consists of 3016 men aged 70–80 years (17) of which 1000 participants were recruited at Uppsala University Hospital. The participants were randomly selected from the population registry and invited by mail. To be eligible for the study, the subjects had to be able to walk without aid and aged 70–80 years. At the clinic visit, participants completed questionnaires about medical history, current medication use, and lifestyle characteristics. Height and weight were measured and blood samples were collected for biochemical analyses. Out of the 1000 individuals included in Uppsala, 86 subjects were excluded from the present study due to serum sample inaccessibility. Accordingly, the total number of serum samples analyzed in this study was 914. Informed consent was obtained from all subjects, and the study was approved by the local ethics committees (ethical approval number; Ups 01–057) and conducted in accordance with the guidelines in The Declaration of Helsinki.

Serum biochemistry

Serum Pi, calcium, albumin, and cystatin C were assessed by standard protocols at the department of clinical chemistry at the Uppsala University Hospital. Serum samples were collected from each individual at a single time point and kept at –70 °C until analysis. Calcium levels presented within this paper were corrected for albumin concentration as follows: calcium (corrected) = calcium – (0.018(albumin – 42)). GFR was indirectly calculated using the following estimate: $GFR = (79.901(cystatin\ C)^{-1.4389})$. PTH levels were analyzed using the Immulite 2000 Intact PTH Assay (Diagnostic Products Corporation, Los Angeles, CA, USA) and 25(OH)D₃ was measured on the Nichols Advantage automated assay system (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Intact FGF23 concentrations were measured using an ELISA according to manufacturer’s protocol (Kainos Laboratories Int’l, Tokyo, Japan) (18). This second-generation, two-site, MAB ELISA has previously been shown to recognize only the biologically active, intact FGF23 protein, but no inactive C-terminal FGF23 fragments. Repeated analysis revealed degradation or measurement error in a small number of samples that was excluded from the subsequent analyses: FGF23 (*n* = 10) and PTH (*n* = 2).

Statistical analysis

All statistical analyses were performed using STATISTICA software (StatSoft Inc., Tulsa, OK, USA). Univariate correlation analyses were used where the Pearson’s correlation coefficient, *r*, measured the degree of linear relationship between the two normally distributed continuous variables. In order to assess how good the predictor variable is in explaining the variability of the target variable, simple linear regression analysis was used. The coefficient of determination, *R*², is the proportion of the variability observed in the data that is explained by the regression line. Multiple linear regression analysis was used to investigate the relationship among the target and several independent predictor variables simultaneously. It was further used to define the relative contributions of the independent

Table 1 Summary of cohort characteristics. Significant differences between the groups stratified for renal function are highlighted in bold.

Variables	Reference range	All subjects	GFR > 60 ml/min	GFR < 60 ml/min
		<i>n</i> = 1000	<i>n</i> = 703	<i>n</i> = 211
Age (years)		75.4 ± 3.1	75.9 ± 3.2	74.4 ± 2.7
Height (mm)		1744 ± 66	1743 ± 67	1745 ± 63
Weight (kg)		80.7 ± 12	80.6 ± 12.2	81.2 ± 11.7
Biochemistry		<i>n</i> = 914	<i>n</i> = 703	<i>n</i> = 211
FGF23 (pg/ml)		44.1 ± 54.1	42.0 ± 58.7	51.2 ± 34.0*
Phosphate (mmol/l)	0.75–1.4	1.12 ± 0.17	1.11 ± 0.16	1.15 ± 0.19*
Calcium (mmol/l)	2.15–2.50	2.46 ± 0.11	2.45 ± 0.10	2.50 ± 0.13†
GFR (ml/min per 1.73 m ²)	> 100	75.9 ± 22.2	84.5 ± 17.0	47.3 ± 10.2†
PTH (pmol/ml)	1.3–7.6	3.8 ± 2.0	3.6 ± 1.7	4.6 ± 2.8†
25(OH)D ₃ (nmol/ml)	15–100	76.5 ± 26	77.7 ± 25.1	77.8 ± 29.6

**P* < 0.01; †*P* < 0.001.

Figure 1 Distribution of intact FGF23 levels in an elderly male population. Median value of FGF23 is 36.6 pg/ml.

variables to the variation in the target variable. The adjusted R^2 was used in the multiple regression analysis and takes into account the number of terms in the model. Comparisons of biochemical changes between groups were performed using ANOVA followed by Tukey's HSD *post hoc* test. For all analyses, standardized β values are presented and a P value <0.05 was considered as statistically significant.

Results

Serum levels of FGF23

The clinical characteristics of the cohort are presented in Table 1. Median value of serum intact FGF23 was 36.6 pg/ml ranging from 0.63 to 957 pg/ml. The distribution of FGF23, excluding five outliers ranging

from 305 to 957 pg/ml, is shown in Fig. 1. FGF23, as well as PTH levels, did not reveal a Gaussian distribution and were therefore transformed into log 10-values for regression analysis.

Univariate analyses of FGF23 and biochemical variables

To determine the co-variance between FGF23 and other serum biochemistries, a general linear regression model was applied. Univariate regression results are presented in Fig. 2a and b. A negative correlation between log FGF23 and eGFR was found ($r = -0.21$; $P < 0.00001$; Fig. 2a), where eGFR explained 4.3% of log FGF23. Additionally, there was a significant and positive correlation between log FGF23 and log PTH ($r = 0.13$; $P < 0.001$; Fig. 2b), indicating that log PTH explained 1.6% of variations in log FGF23. No significant correlation between log FGF23 and Pi ($P = 0.75$), calcium ($P = 0.12$) or 25(OH)D3 ($P = 0.09$) was observed. Notably, the $[Ca \times Pi]$ product is a predictor of FGF23 in CKD (19). We did not find any significant correlation between log FGF23 and $[Ca \times Pi]$ product in our cohort ($P = 0.93$).

Multivariate analyses of FGF23 and biochemical variables

To establish the relative contribution of explanatory variable for log FGF23, we performed a multivariate regression analysis with Pi, calcium, eGFR, log PTH, and 25(OH)D3 as independent variables. Notably, eGFR ($\beta = -0.19$, $P < 0.00001$) and log PTH ($\beta = 0.08$, $P = 0.020$) remained as significant explanatory variables for log FGF23. Pi, calcium, and 25(OH)D3 similarly failed to influence log FGF23 in this model (Table 2).

We further analyzed subjects stratified for renal function. Multivariate regression analysis in subjects

Figure 2 Univariate regression analysis of log FGF23 co-variance with (a) eGFR and (b) log PTH.

Table 2 Linear univariate and multivariate regression analysis of all study subjects and log fibroblast growth factor-23 (FGF23) levels as dependent variable.

<i>n</i> =914	<i>r</i> value	<i>P</i> value	β value	<i>P</i> value
Phosphate	−0.011	0.75	−0.039	0.27
Calcium	0.051	0.12	0.012	0.74
eGFR	−0.21	<0.00001	−0.19	<0.000001
log PTH	0.13	<0.001	0.080	<0.05
25(OH)D3	−0.058	0.094	−0.055	0.10

Significant *P* values are bolded.

with eGFR > 60 ml/min (*n* = 703) revealed that eGFR (β = −0.09; *P* = 0.022) and log PTH (β = 0.08; *P* = 0.036) remained as explanatory variables for log FGF23 (Table 3). In contrast, eGFR (β = −0.35; *P* < 0.0001) was the only predictor of log FGF23 in subjects with eGFR < 60 ml/min (Table 4). All biochemical variables, except 25(OH)D3, were statistically different in the two groups stratified for renal function (Table 1).

Multivariate analyses of PTH and biochemical variables

Since we found an association between FGF23 and PTH, we sought to analyze other variables associated with log PTH. Multivariate regression analysis with Pi, calcium, GFR, 25(OH)D3, and log FGF23 as independent variables revealed that log FGF23, (β = 0.08; *P* = 0.020), Pi (β = −0.08; *P* = 0.026), and eGFR (β = −0.17; *P* < 0.00001) were explanatory variables for log PTH.

Discussion

In the study cohort described herein, we found an independent and positive association between serum FGF23 and PTH. This association has not previously been described in subjects without CKD. The sample size in the current study is also of greater magnitude than any previous published data on serum FGF23 levels, increasing the probability of detecting such an association. Our data could imply a physiological co-regulation of FGF23 and PTH *in vivo*. Further support for this hypothesis arose from our recent observations that FGF23 potently

Table 3 Linear univariate and multivariate regression analysis of subjects with glomerular filtration rate (GFR) > 60 ml/min and log fibroblast growth factor-23 (FGF23) as dependent variable.

<i>n</i> =703	<i>r</i> value	<i>P</i> value	β value	<i>P</i> value
Phosphate	−0.006	0.87	−0.034	0.40
Calcium	0.062	0.10	0.046	0.26
eGFR	−0.10	<0.01	−0.090	<0.05
log PTH	0.10	<0.01	0.082	<0.05
25(OH)D3	−0.046	0.24	−0.043	0.27

Significant *P* values are bolded.

Table 4 Linear univariate and multivariate regression analysis of subjects with estimated glomerular filtration rate (eGFR) < 60 ml/min and log fibroblast growth factor-23 (FGF23) as dependent variable.

<i>n</i> =211	<i>r</i> value	<i>P</i> value	β value	<i>P</i> value
Phosphate	−0.089	0.20	−0.099	0.19
Calcium	0.082	0.24	−0.065	0.37
eGFR	−0.34	<0.000001	−0.35	<0.0001
log PTH	0.11	0.11	−0.27	0.73
25(OH)D3	0.098	0.18	−0.10	0.14

Significant *P* values are bolded.

decreases PTH mRNA expression and protein secretion *in vitro*, using primary isolates of bovine parathyroid cells (5). Additionally, one preliminary report showed that injection of PTH in mice increased serum FGF23 levels (20). Thus, it is possible that small and immediate changes in serum FGF23 alter PTH secretion and vice versa, supporting the idea of a bone–parathyroid axis required for maintenance of a proper mineral ion homeostasis.

We also found that eGFR was significantly associated with FGF23, including subjects with eGFR > 60 ml/min. It is previously well established that severely impaired renal function causes increments in serum FGF23, in part due to chronic hyperphosphatemia, but also due to decreased renal clearance of FGF23 (11, 12). Because our study subjects have normal serum Pi, we conclude that decreased renal clearance of FGF23 likely proceeds abnormalities in calcium/Pi balance present at later stages of CKD. Although a highly significant association, the correlation coefficient for log FGF23 and eGFR is relatively low. Thus, eGFR cannot be used as a reliable predictor of serum FGF23 in patients with normal renal function or in early CKD. Since all participants of the current study were males and displayed a narrow age span, we cannot rule out the possibility that increased age or gender differences contribute to elevated serum FGF23 in some subjects, even when adjusted for renal function. However, serum FGF23 in previous measured cohorts of healthy controls or CKD subjects did not vary significantly with age or gender (10, 13). Finally, the FGF23 values in the current cohort are slightly higher than a previously established reference range in 104 healthy controls for the intact FGF23 ELISA used herein (28.9 ± 1.1 pg/ml; mean $\pm$ s.d.) (18). This discrepancy can most likely be attributed to a lower mean eGFR in the subjects used within the present study.

We failed to detect any significant correlation between FGF23 and Pi. Notably, to our knowledge, no correlation between FGF23 and Pi has previously been described in subjects with normal renal function without manipulating dietary Pi intake (21–23). The fact that PTH, but not FGF23, correlates to Pi in our study may also imply that Pi induces changes in serum PTH more rapidly than in FGF23. This is supported by a recent study where dietary Pi load resulted in a rapid increase in PTH, whereas FGF23 remained unchanged during the first 8 h (21). It would be of interest to determine the relationship between FGF23

and dietary Pi intake in the cohort used herein, however, these data are unavailable.

FGF23 decreases serum calcitriol *in vivo* (1, 2) and there is an inverse correlation between FGF23 and calcitriol in CKD (14). Therefore, a limitation of this study is the absence of 1,25(OH)₂D₃ measurements. However, we found a trend that 25(OH)D₃ was negatively correlated to FGF23, although above the threshold of significance.

In summary, serum FGF23 is independently associated with PTH and renal function in a population-based cohort of elderly men. Our study implies a possible co-regulation of FGF23 and PTH and sheds further light on the regulation of FGF23 in states of normophosphatemia.

Acknowledgements

We would like to thank Anna-Lena Johansson for technical assistance. This work was supported by the Novo Nordisk Foundation, the Swedish Kidney Foundation, and the Swedish Society of Medicine. T E L and Ö L received lecture fees. The other authors have nothing to declare.

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Received 7 August 2007

Accepted 9 September 2007