Fibroblast growth factor 23 and markers of mineral metabolism in individuals with preserved renal function

DHAYAT, Nasser A, et al.

Abstract

Fibroblast growth factor 23 (FGF23) is a bone-derived hormone that regulates phosphate homeostasis. Circulating FGF23 is elevated in chronic kidney disease (CKD) and independently associated with poor renal and cardiovascular outcomes and mortality. Because the study of FGF23 in individuals with normal renal function has received little attention, we examined in a large, population-based study of 1128 participants the associations of FGF23 with markers of mineral metabolism and renal function. The median estimated glomerular filtration rate (eGFR) of the cohort was 105 ml/min per 1.73 m(2), and the median plasma FGF23 was 78.5 RU/ml. FGF23 increased and plasma 1,25-dihydroxyvitamin D3 decreased significantly below an eGFR threshold of 102 and 99 ml/min per 1.73 m(2), respectively. In contrast, plasma parathyroid hormone increased continuously with decreasing eGFR and was first significantly elevated at an eGFR of 126 ml/min per 1.73 m(2). On multivariable analysis adjusting for sex, age, body mass index, and GFR, FGF23 was negatively associated with 1,25-dihydroxyvitamin D3, and urinary absolute and fractional calcium […]

Reference


DOI : 10.1016/j.kint.2016.04.024
PMID : 27370409

Available at:
http://archive-ouverte.unige.ch/unige:89458

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Fibroblast growth factor 23 and markers of mineral metabolism in individuals with preserved renal function

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Fibroblast growth factor 23 (FGF23) is a bone-derived hormone that regulates phosphate homeostasis. Circulating FGF23 is elevated in chronic kidney disease (CKD) and independently associated with poor renal and cardiovascular outcomes and mortality. Because the study of FGF23 in individuals with normal renal function has received little attention, we examined in a large, population-based study of 1128 participants the associations of FGF23 with markers of mineral metabolism and renal function. The median estimated glomerular filtration rate (eGFR) of the cohort was 105 ml/min per 1.73 m², and the median plasma FGF23 was 78.5 RU/ml. FGF23 increased and plasma 1,25-dihydroxyvitamin D3 decreased significantly below an eGFR threshold of 102 and 99 ml/min per 1.73 m², respectively. In contrast, plasma parathyroid hormone increased continuously with decreasing eGFR and was first significantly elevated at an eGFR of 126 ml/min per 1.73 m². On multivariable analysis adjusting for sex, age, body mass index, and GFR, FGF23 was negatively associated with 1,25-dihydroxyvitamin D3, and urinary absolute and fractional calcium excretion but not with serum calcium or parathyroid hormone. We found a positive association of FGF23 with plasma phosphate, but no association with urinary absolute or fractional phosphate excretion and, unexpectedly, a positive association with tubular maximum phosphate reabsorption/GFR. Thus, in the absence of CKD, parathyroid hormone increases earlier than FGF23 when the eGFR decreases. The increase in FGF23 occurs at a higher eGFR threshold than previously reported and is closely associated with a decrease in 1,25-dihydroxyvitamin D3. We speculate that the main demonstrable effect of FGF23 in the setting of preserved renal function is suppression of 1,25-dihydroxyvitamin D3 rather than stimulation of renal phosphate excretion.


KEYWORDS: calcium; FGF23; phosphate; PTH; TmP/GFR

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Fibroblast growth factor 23 (FGF23) is a hormone secreted by osteocytes in the bone in response to dietary phosphate loading or an increase in 1,25-dihydroxyvitamin D₃.¹ Binding of FGF23 to its coreceptor klotho increases the affinity of FGF23 for ubiquitously expressed FGF receptors. Klotho is mainly expressed in the kidney, brain, and parathyroid gland and thus is responsible for the cellular specificity of FGF23 action. FGF23 induces phosphaturia by downregulation of proximal tubular NaPi-IIa and -IIc transporters, reduces 1,25-dihydroxyvitamin D₃ by downregulation of 1-α-hydroxylase and upregulation of 24-hydroxylase, and suppresses parathyroid hormone (PTH) secretion.¹ In healthy individuals, when phosphate intake is high and sustained, FGF23 levels rise, leading to increased phosphaturia and downregulation of calcitriol synthesis, thereby limiting phosphate absorption, with the net result of negative external phosphate balance.² In chronic kidney disease (CKD), levels of FGF23 increase, presumably to maintain phosphate balance as an adaptive response in the setting of decreased klotho levels and reduced renal excretory capacity for phosphate. As the glomerular filtration rate (GFR) decreases, levels of FGF23 rise progressively and to very high levels.³ Increased FGF23 is independently...

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Received 30 December 2015; revised 21 April 2016; accepted 28 April 2016; published online 28 June 2016

648

associated with poor renal and cardiovascular outcomes, left ventricular hypertrophy, and mortality in CKD patients.\textsuperscript{3–6} The highest eGFR values are found in patients on dialysis. In a large prospective study following incident dialysis patients, high FGF23 was independently associated with increased mortality.\textsuperscript{7}

In contrast to CKD patients, the study of FGF23 in individuals with normal renal function has received much less attention so far. Studies assessing the role of FGF23 in non-CKD patients have been restricted to specialized subgroups of individuals and/or limited by the mineral metabolism analyte phenotype available.\textsuperscript{8–11} Thus, although much has been learned about FGF23 pathophysiology by the investigation of CKD patients in the past years, critical questions about the physiological role of FGF23 under normal renal function remain unanswered. To obtain more insight into FGF23 physiology, we conducted a cross-sectional analysis of markers of mineral metabolism in a large, multicenter, population-based cohort in Switzerland.

RESULTS

Characteristics of the study population

From December 2009 to March 2013, 1128 participants were recruited in the SKIPOGH (Swiss Kidney Project on Genes in Hypertension) study. Inclusion and exclusion criteria and study details are provided in the Methods section. Demographic and anthropometric characteristics of the 1010 individuals who were included in the final analysis are shown in Table 1. The mean ± SD age of the cohort was 45.5 ± 16.9 years; 47.4% of participants were men. The median eGFR\textsubscript{cr-cys} (Creatinine-Cystatin C Equation CKD-EPI 2009; eGFR \textsuperscript{cr-cys}, creatinine-cystatin C Equation CKD-EPI 2012; eGFR\textsubscript{cr}, creatinine clearance subgroups)

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<th>Characteristics</th>
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<td>Age, yr</td>
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<td>Body mass index, kg/m\textsuperscript{2}</td>
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<td>Systolic blood pressure, mm Hg</td>
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<tr>
<td>Diastolic blood pressure, mm Hg</td>
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<tr>
<td>Hypertension</td>
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<tr>
<td>Diabetes</td>
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<td>Creatinine, µmol/l</td>
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<tr>
<td>Cystatin C, mg/l</td>
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<td>0.738 ± 0.126</td>
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<tr>
<td>eGFR\textsubscript{cr-cys}, ml/min per 1.73 m\textsuperscript{2} BSA</td>
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<td>98.2 (87.0–109.9)</td>
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<td>FGF23, RU/ml</td>
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<td>PTH, pg/ml</td>
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<td>25-OH-vitamin D\textsubscript{3}, nmol/l</td>
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<td>91.0 (70.0–116.0)</td>
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<td>Phosphate, mmol/l</td>
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<tr>
<td>Calcium corrected, mmol/l</td>
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<td>3.23 ± 0.09</td>
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<td>Phosphaturia, mmol/24 hr</td>
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<td>25.3 (20.3–32.1)</td>
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<td>Fractional excretion of phosphate, %</td>
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<td>16.5 (12.9–20.9)</td>
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<td>TmP/GFR, mmol/l</td>
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<td>Calcium, mmol/24 hr</td>
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<td>Fractional excretion of calcium, %</td>
<td>967</td>
<td>1.76 (1.10–2.68)</td>
</tr>
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</table>

Table 1 | Baseline characteristics of the study population

Mineral metabolism parameters in relation to eGFR

We next stratified the cohort into groups of descending eGFR\textsubscript{cr-cys} values to assess the association of mineral metabolism parameters with renal function. There was a gradual increase in mean age and body mass index (BMI) with decreasing eGFR (Table 2). Consistent with previous reports, urine fractional excretion of phosphate (FE\textsubscript{Pi}) increased, the ratio of tubular maximum reabsorption of phosphate (TmP) to GFR (TmP/GFR) decreased, and 24-hour urinary phosphate excretion decreased with decreasing eGFR (Table 2).\textsuperscript{16–18} Interestingly, alterations in plasma PTH, FGF23, and 1,25-dihydroxyvitamin D\textsubscript{3} were already evident in the normal eGFR range. In the eGFR subgroup of 110 to 119.9 ml/min per 1.73 m\textsuperscript{2}, plasma PTH was already significantly increased compared with the eGFR subgroup ≥120 ml/min per 1.73 m\textsuperscript{2}. A first increase of plasma FGF23 was evident in the eGFR subgroup of 100 to 109.9 ml/min per 1.73 m\textsuperscript{2}; plasma 1,25-dihydroxyvitamin D\textsubscript{3} was first significantly reduced in the eGFR subgroup of 90 to 99.9 ml/min per 1.73 m\textsuperscript{2}. A similar pattern with an earlier increase in plasma PTH than FGF23 was observed when the cohort was stratified into groups of...
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<th>eGFR 90–99.9</th>
<th>eGFR 80–89.9</th>
<th>eGFR &lt;80</th>
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<td>238</td>
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<td>226</td>
<td>238</td>
<td>188</td>
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<td>75</td>
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<tr>
<td>eGFRcr, ml/min per 1.73 m² BSA</td>
<td>119</td>
<td>226</td>
<td>238</td>
<td>189</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>eGFRcr-cys, ml/min per 1.73 m² BSA</td>
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<td>226</td>
<td>238</td>
<td>189</td>
<td>75</td>
<td>75</td>
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<tr>
<td>Creatinine clearance corrected, ml/min per 1.73 m² BSA</td>
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<td>205</td>
<td>221</td>
<td>178</td>
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<tr>
<td>FGF23, RU/ml</td>
<td>119</td>
<td>226</td>
<td>238</td>
<td>189</td>
<td>75</td>
<td>75</td>
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<tr>
<td>PTH, pg/ml</td>
<td>117</td>
<td>226</td>
<td>237</td>
<td>188</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>25-OH-vitamin D₃, nmol/l</td>
<td>117</td>
<td>226</td>
<td>237</td>
<td>188</td>
<td>75</td>
<td>75</td>
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<tr>
<td>1,25-dihydroxyvitamin D₃, pmol/l</td>
<td>109</td>
<td>209</td>
<td>232</td>
<td>181</td>
<td>71</td>
<td>71</td>
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<tr>
<td>Phosphate, mmol/l</td>
<td>119</td>
<td>226</td>
<td>237</td>
<td>189</td>
<td>75</td>
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<tr>
<td>Calcium, corrected, mmol/l</td>
<td>119</td>
<td>226</td>
<td>238</td>
<td>188</td>
<td>75</td>
<td>75</td>
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<tr>
<td>Urine values</td>
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<tr>
<td>Phosphaturia, mmol/24 h</td>
<td>113</td>
<td>218</td>
<td>233</td>
<td>181</td>
<td>72</td>
<td>72</td>
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<td>Fractional excretion of phosphate, %</td>
<td>114</td>
<td>218</td>
<td>232</td>
<td>181</td>
<td>72</td>
<td>72</td>
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<td>TmP/GFR, mmol/l</td>
<td>114</td>
<td>218</td>
<td>232</td>
<td>181</td>
<td>72</td>
<td>72</td>
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<tr>
<td>Calcium, mmol/24 h</td>
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<td>217</td>
<td>231</td>
<td>180</td>
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<tr>
<td>Fractional excretion of calcium, %</td>
<td>113</td>
<td>217</td>
<td>231</td>
<td>180</td>
<td>74</td>
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</table>

1,25-dihydroxyvitamin D₃, 1,25-dihydroxyvitamin D₃, 25-OH-VitD₃, 25-hydroxyvitamin D₃, ANOVA, analysis of variance; BSA, body surface area; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFRcr, estimated glomerular filtration rate using creatinine; eGFRcr-cys, estimated glomerular filtration rate using the combination of creatinine and cystatin C; FGF23, fibroblast growth factor 23; PTH, parathyroid hormone; GFR, glomerular filtration rate; h, hour; TmP/GFR, ratio of tubular maximum resorption of phosphate to glomerular filtration rate; yr, year.

The number of individuals is indicated for each characteristic stratified by eGFRcr-cys subgroups. Continuous variables are indicated by their mean ± SD or by their median (25th–75th percentile). Between-group differences are determined by 1-way ANOVA (Welch) not assuming equal variances. If null hypothesis was rejected, pairwise comparisons of the eGFRcr-cys subgroups were performed using t tests with pooled SD and alpha error adjustment by the method of Benjamini-Hochberg.

*P < 0.05 compared with eGFRcr-cys subgroup 1 level lower.
†P < 0.05 compared with eGFRcr-cys subgroup 2 levels lower.
‡P < 0.05 compared with eGFRcr-cys subgroup 3 levels lower.
§P < 0.05 compared with eGFRcr-cys subgroup 4 levels lower.
¶P < 0.05 compared with eGFRcr-cys subgroup 5 levels lower.
decreasing eGFRcr (creatinine equation CKD-EPI 2009) values (Supplementary Table S1) or creatinine clearance (Supplementary Table S2).

When plotting plasma FGF23 versus eGFRcr-cys using a quadratic spline function, the increase in log-transformed plasma FGF23 became significant at an eGFR of 102.3 ml/min per 1.73 m² (Figure 1a). This threshold represents the highest eGFR at which the 95% confidence interval (CI) for log plasma FGF23 did not overlap anymore with the 95% CI of log plasma FGF23 at any higher eGFR. Using the same approach, we observed a significant decrease in plasma 1,25-dihydroxyvitamin D₃ at an eGFR of 102.3 ml/min per 1.73 m² (Figure 1b) and an increase in plasma PTH at an eGFRcr-cys of 126.3 ml/min per 1.73 m² (Figure 1c). An increasing plasma PTH was also the earliest sign of altered mineral metabolism when GFR was estimated by eGFRcr (Supplementary Figure S1) or by creatinine clearance (Supplementary Figure S2).

**Mineral metabolism parameters in relation to 25-hydroxyvitamin D₃ status**

The 25-hydroxyvitamin D₃ status was shown previously to influence relative increases in PTH and FGF23 in patients with stage 3 CKD. To study the impact of plasma 25-hydroxyvitamin D₃ on mineral metabolism parameters in individuals with preserved renal function, we divided our cohort into quartiles of plasma 25-hydroxyvitamin D₃ status (Table 3). Compared with individuals in the lowest plasma 25-hydroxyvitamin D₃ quartile, individuals in the highest plasma 25-hydroxyvitamin D₃ quartile were younger, had a lower BMI, a lower PTH level, a higher plasma 1,25-dihydroxyvitamin D₃ level, higher plasma phosphate and plasma calcium levels, and higher absolute and fractional calcitriol, but did not differ in GFR, plasma FGF23, absolute or fractional phosphaturia, or TmP/GFR.

To assess the role of plasma 25-hydroxyvitamin D₃ on the increase in plasma FGF23 and PTH with decreasing GFR, we rebuilt Figure 1, adjusting for plasma 25-hydroxyvitamin D₃. As shown in Supplementary Figure S3, even after adjustment for plasma 25-hydroxyvitamin D₃ status, plasma PTH increases significantly earlier than plasma FGF23.

**Association analyses**

We next performed univariable association analyses of plasma FGF23 by mixed-effects linear regression models, taking family, center, and day of blood sampling as random effects into account (Table 4, model 1). The strongest negative correlate of plasma FGF23 was plasma 1,25-dihydroxyvitamin D₃ (linear β: −0.00312, P < 0.001; quadratic β: 2.1 × 10⁻⁵, P = 0.026). In addition, plasma FGF23 correlated positively with plasma phosphate (β: 0.32701, P < 0.001) and plasma PTH (β: 0.002768, P = 0.014) and negatively with plasma 25-hydroxyvitamin D₃ (linear β: −0.00204, P = 0.0079; quadratic β: 3.2 × 10⁻⁵, P = 0.084). As expected, renal function was strongly negatively associated with plasma FGF23: eGFRcr (linear β: −0.004281, P < 0.001; quadratic β: 8.2 × 10⁻⁵, P < 0.0111), eGFRcr-cys (linear β: −0.005687, P < 0.001; quadratic β: 7.4 × 10⁻⁵, P = 0.053), and creatinine clearance (linear β: −0.002939, P < 0.001, quadratic β: 5.0 × 10⁻⁵, P = 0.001). Interestingly, we observed also an inverse association of plasma FGF23 with urinary calcium excretion (linear β: −0.042465, P < 0.001; quadratic β: 0.005725, P = 0.0016), and fractional calcium excretion (β: −0.02251, P = 0.059), but no association with plasma calcium (β: 0.24939, P = 0.14). There was no significant association of plasma FGF23 with 24-hour urinary phosphate excretion or FEPi, but surprisingly a positive association with TmP/eGFR (β: 0.20217, P = 0.0091).

We then conducted a multivariable analysis taking family, center, and calendar day of blood sampling as random effects into account and adjusting for sex, age, BMI, and GFR (Table 4). GFR was approximated as eGFRcr (model 2) or eGFRcr-cys (model 3) or measured creatinine clearance (model 4). After adjustment, plasma 1,25-dihydroxyvitamin...

In line with a previous study in CKD patients, we found that plasma PTH levels are also affected by plasma 25-hydroxyvitamin D3 status in individuals with preserved renal function. In addition, our data strongly indicate that increasing plasma PTH is the earliest sign of altered mineral metabolism when GFR decreases, well before the onset of CKD and independent of plasma 25-hydroxyvitamin D3 status. This finding is in apparent contradiction to an earlier large cohort study that reported that FGF23 becomes increased earlier than PTH in the course of CKD. However, there are significant differences between the 2 cohorts that may explain some of the discrepancies. Our cohort consists of mostly healthy individuals without CKD (only 1.2% with an eGFR <60 ml/min per 1.73 m2), whereas in the CRIC (Chronic Renal Insufficiency Cohort) study, 89.5% of participants had an eGFR <60 ml/min per 1.73 m2. Significant differences between the 2 cohorts also exist with respect to comorbidities and ethnicity of participants.

Although plasma PTH increases clearly earlier than plasma FGF23 when GFR decreases in healthy individuals of European ancestry, additional studies are needed to assess the generalizability of our findings.

### DISCUSSION

We performed a thorough analysis of markers of calcium and phosphate metabolism in a population-based cohort of 1128 adult individuals, with the majority of them having preserved renal function. To our knowledge, this is the largest observational study of this kind with such a detailed phenotype. Although the study partly corroborates previous work, it reveals some disparate results and several very important novel findings. In individuals with preserved renal function, plasma FGF23 correlates strongly and inversely with plasma 1,25-dihydroxyvitamin D3 and changes in plasma FGF23 and 1,25-dihydroxyvitamin D3 occur at a much higher GFR than previously reported. FGF23 also correlates weakly negatively with plasma 25-hydroxyvitamin D3, suggesting that FGF23 may stimulate Cyp24A1-mediated inactivation of 25-hydroxyvitamin D3 to 24,25-hydroxyvitamin D3.

In line with a previous study in CKD patients, we found that plasma PTH levels are also affected by plasma 25-hydroxyvitamin D3 status in individuals with preserved renal function. In addition, our data strongly indicate that increasing plasma PTH is the earliest sign of altered mineral metabolism when GFR decreases, well before the onset of CKD and independent of plasma 25-hydroxyvitamin D3 status. This finding is in apparent contradiction to an earlier large cohort study that reported that FGF23 becomes increased earlier than PTH in the course of CKD. However, there are significant differences between the 2 cohorts that may explain some of the discrepancies. Our cohort consists of mostly healthy individuals without CKD (only 1.2% with an eGFR <60 ml/min per 1.73 m2), whereas in the CRIC (Chronic Renal Insufficiency Cohort) study, 89.5% of participants had an eGFR <60 ml/min per 1.73 m2. Significant differences between the 2 cohorts also exist with respect to comorbidities and ethnicity of participants. Although plasma PTH increases clearly earlier than plasma FGF23 when GFR decreases in healthy individuals of European ancestry, additional studies are needed to assess the generalizability of our findings.
### Table 4 | Associations between log-transformed plasma FGF23, as dependent variable, with basic demographic and plasma and urinary parameters of calcium phosphate metabolism as predictor variables

<table>
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<th>Predictor variable</th>
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<td>Assessment of renal function</td>
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<td>eGFRc, ml/min per 1.73 m² BSA</td>
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<td>−0.00204</td>
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<td>Phosphate, mmol/l</td>
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<td>0.32701</td>
<td>0.15639–0.50727</td>
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<td>1000</td>
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<td>−0.00067</td>
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<td>−0.00798 to 0.003</td>
<td>0.40</td>
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<td>−0.00357</td>
<td>−0.00939 to 0.00205</td>
<td>0.22</td>
<td>909</td>
<td>−0.00295</td>
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(Continued on next page)
Although there was a clear association of plasma FGF23 with plasma phosphate, we found no association of plasma FGF23 with FEPi but a positive association of plasma FGF23 with TmP/GFR. This is an unexpected and surprising finding for a hormone that is considered the prototype of a phosphatonin. FGF23 is less sensitive than TmP/GFR for the detection of alterations in renal phosphate handling (despite the exact same input terms), thus a weak association of plasma FGF23 with FEPi may be missed, but a *bona fide* phosphatonin should decrease (and not increase) TmP/GFR. However, the observation of a positive association of FGF23 with TmP/GFR at a high GFR is not an unprecedented finding and has been reported by other investigators using both intact and C-terminal FGF23 assays. Plasma phosphate and tubular reabsorption of phosphate (TRP) are the 2 parameters used for TmP/GFR estimation. Given the strong positive association of plasma FGF23 with plasma phosphate and the lack of an association with FEPi (which is equal to 1 – TRP), it is evident that plasma phosphate is driving the positive association of plasma FGF23 with TmP/GFR in the calculation. Elevated plasma phosphate likely triggers FGF23 release, even in the absence of CKD. Interestingly, however, our data indicate that elevated plasma FGF23 in our cohort is associated with low plasma 1,25-dihydroxyvitamin D₃ but not with increased renal phosphate excretion. This suggests that thresholds for inhibition of 1,25-dihydroxyvitamin D₃ synthesis and induction of phosphaturia by FGF23 may not be the same. Such a concept of a differential FGF23 effect in the proximal tubule would be in line with experimental data in mice that revealed different pathways for the inhibition of 1,25-dihydroxyvitamin D₃ synthesis and phosphate reabsorption. Thus, in the setting of preserved renal function, the induction of phosphaturia by FGF23 may require additional factors or simply higher FGF23 levels.

In contrast to FEPi and 24-hour phosphate excretion, FECa and 24-hour calcium excretion were strongly and inversely associated with plasma FGF23. It is clear that the original view of FGF23 as a pure phosphaturic hormone is too limited. In mice, FGF23 was recently shown to stimulate renal calcium reabsorption via an increase in apical membrane abundance of transient receptor potential vanilloid-5 in the distal convoluted tubule. Calcium intake directly influenced FGF23 secretion, independently of PTH and 1,25-dihydroxyvitamin D₃ in mice and rats. In humans, in both healthy volunteers and dialysis patients, short-term changes in plasma calcium had no effect on circulating FGF23 levels. Chronically, however, both high dialysate calcium as well as high calcium intake were associated with higher FGF23 levels in dialysis patients. Whether the association of FGF23 with reduced urinary calcium excretion observed in our study is the result of a direct FGF23 effect on the kidney or secondary to the FGF23-mediated reduction in 1,25-dihydroxyvitamin D₃ cannot be answered by our cross-sectional study and remains to be tested using longitudinal data. Taken together, however, our data suggest that in individuals with normal renal function, plasma FGF23 is

### Table 4 (Continued)

<table>
<thead>
<tr>
<th>Predictor variable</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
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<td>N</td>
<td>β</td>
<td>95% CI</td>
<td>N</td>
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<td>Calcium, mmol/l</td>
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<td>Fractional Calcium, %</td>
<td>Linear</td>
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<td>0.0089–0.0095</td>
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</table>

FGF23, fibroblast growth factor 23; 25-OH, 25-hydroxyvitamin D₃; PTH, parathyroid hormone; TmP/GFR, ratio of tubular maximum reabsorption of phosphate to glomerular filtration rate.
associated with reduced plasma 1,25-dihydroxyvitamin D₃ and reduced renal calcium excretion but not with increased renal phosphate excretion.

Our study has several limitations. The study was restricted to participants of European descent, and the study design was cross-sectional; thus, only associations and no causal relationships can be inferred. No direct GFR measurements based on exogenous filtration markers were available. GFR was either approximated as creatinine clearance or estimated by the CKD-EPI equation or by the combined CKD-EPI-cystatin C equation. The latter was shown to be superior for GFR estimation in CKD and non-CKD patients compared with purely creatinine- or cystatin C-based formulas. Another limitation is that FGF23 was measured with the second-generation C-terminal assay for FGF23, which detects both intact FGF23 and C-terminal fragments thereof and thus may overestimate "bioactive" FGF23. However, compared with intact FGF23 assays, the C-terminal assay has the advantage of lower intra- but higher interindividual variability, higher preanalytical stability, and the lack of diurnal variation. In addition, the C-terminal FGF23 assay used in our study is well validated and has been widely used in cross-sectional as well as clinical outcome studies, both in CKD and non-CKD patients. Finally, the same assay was used for all the individuals, so comparison within the cohort is valid. Despite these limitations, our study clearly expands the current knowledge on mineral metabolism in humans with preserved renal function. Yet, longitudinal observational studies and intervention trials are needed to fully understand the complex interplay between FGF23, PTH, and 1,25-dihydroxyvitamin D₃ and the role of these hormones in clinical outcomes.

METHODS

Study population

The SKIPOGH study is a multicenter, family-based, cross-sectional study exploring the role of genes and kidney hemodynamics in blood pressure regulation and kidney function in the general adult population. A detailed description of the methods was reported recently. Briefly, from December 2009 to March 2013, 1128 adult participants were recruited in the 2 regions of Berne and Geneva and in the city Lausanne in Switzerland. Inclusion criteria were as follows: (i) a minimum age of 18 years; (ii) European ancestry; (iii) having at least 1 and ideally 3 first-degree family members willing to participate; and (iv) providing a written informed consent. The SKIPOGH study adhered to the Declaration of Helsinki and was approved by the institutional ethics committees of each participating university hospital.

In 1122 of a total of 1128 SKIPOGH participants, plasma samples were available for FGF23 determination. Participants were excluded from the final analysis for the following reasons: (i) hyperparathyroidism or parathyroidectomy (n = 2); (ii) chronic liver disease (n = 1); (iii) active malignant diseases (n = 10); (iv) pregnancy (n = 1); (v) intake of the following drugs: over-the-counter or prescribed calcium and/or vitamin D supplements (n = 22), bisphosphonates (n = 2), glucocorticoids or mineralocorticoids (n = 5), antiepileptic agents (n = 2), loop diuretics (n = 6), thiazide diuretics (n = 63), potassium sparing diuretics (n = 5), and desmopressin (n = 1). A total of 1010 participants (89.5%) of 269 distinct families were included in the final analysis.

Data collection and measurements

A 24-hour urine sample was collected separately for day and night. Night urine included only urine voided during sleep time and the first morning urine void. We followed the recommendation to use the urinary creatinine excretion as the criterion for completeness of 24-hour urine collections in individuals with normal renal function. Therefore, 2.5 and 97.5 centiles of the 24-hour creatinine excretion were calculated for each reference individual using a linear regression model with sex, BMI, and age as predictor variables recently published with data derived from the cross-sectional Swiss Salt Study (SSS). The SSS includes a random population-based, cross-sectional sample of 1535 individuals, and the linear regression model was validated in 994 participants from the SKIPOGH study. In this study, completeness of 24-hour urine collections was assumed if the total 24-hour creatinine excretion was within centiles 2.5 and 97.5, as described previously.

Fasting morning electrolyte, blood glucose, albumin, and renal function tests were measured by standard clinical laboratory methods at each center. Plasma FGF23, PTH, 25-hydroxyvitamin D₃, and 1,25-dihydroxyvitamin D₃ were determined centrally for all study participants as single measurements. Plasma FGF23 was measured in the laboratory of TECO Medical AG (Sissach, Switzerland) using the second-generation C-terminal assay (Immunoassays, San Clemente, CA). The lower detection limit of the assay was 1.5 relative units/ml, and the intra- and interassay coefficients of variation were 1.4% to 2.4% and 2.4% to 4.7%, respectively. Plasma PTH, 25-hydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₃ were measured at the Center of Laboratory Medicine (Inselspital, Berne, Switzerland) as follows: plasma intact PTH by electrochemiluminescence immunoassay on a Roche Modular E170 analyzer (Roche Diagnostics AG, Rotkreuz, Switzerland), plasma 25-hydroxyvitamin D₃ by a direct, competitive chemiluminescence immunoassay on a LIAISON Analyzer (DiaSorin S.p.A., Saluggia, Italy), and plasma 1,25-dihydroxyvitamin D₃ by a competitive enzyme immunoassay on a Multilabel Counter Victor 3 (PerkinElmer, Inc., Waltham, MA). Intra- and interassay coefficients of variation are 1.1% to 2.0% and 2.5% to 3.4% for plasma PTH, 4.1% to 5.8% and 6.6% to 7.1% for plasma 25-hydroxyvitamin D₃, and <8% to <10% for plasma 1,25-dihydroxyvitamin D₃, respectively.

The creatinine-based CKD-EPI 2009 equation was used to estimate the eGFRcr, and the creatinine-cystatin C-based equation CKD-EPI 2012 was used to estimate eGFRcr-cys. In addition, the GFR was calculated from the 24-hour urine collection corrected for the body surface area (BSA). We excluded 96 urine samples from the calculation of GFR due to inaccurate urine collection or missing plasma or urine creatinine values.

We calculated the fractional excretion of phosphate by the formula FEPi (%) = [(fasting night urine phosphate (mmol/l) × fasting plasma creatinine (µmol/l)) / (fasting plasma phosphate (mmol/l) × fasting night urine creatinine (µmol/l))] × 100. In case of plasma albumin ≤40 g/l, we calculated the corrected plasma calcium by the formula: Cacorr (µmol/l, %) = plasma calcium measured (µmol/l) + 0.025 × [40 – plasma albumin (g/l)]. Fractional excretion of calcium was calculated by the formula FECa (%) = [(fasting night urine calcium (µmol/l) × fasting plasma creatinine (µmol/l)) / (corrected fasting plasma calcium (µmol/l) × 0.5 × fasting night


655

NA Dhayat et al.: FGF23 in individuals with normal renal function
urine creatinine (μmol/l) × 100. TRP was calculated by the formula: TRP (%) = (100 − FePi [%]). TmP/GFR (in mmol/l) was calculated with the algorithm derived by Kenny and Glen. If TRP was ≤86%, then the formula TmP/GFR (mmol/l) = TRP × plasma phosphate (mmol/l) was used. If TRP was >0.86%, then the formula TmP/GFR (mmol/l) = (0.3 × TRP)/(1 − 0.8 × TRP) × (plasma phosphate [mmol/l]) was used.

Diabetes was defined as reported, treated, or fasting glycaemia ≥7 mmol/l. Hypertension was defined as either systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or the use of antihypertensive medications.

Statistical analysis
All statistical analyses were conducted using R software, version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). The shape of the distribution of each continuous variable was visually inspected and the square root, log, or inverse transformations were applied to better ensure a normal approximation of the residuals for statistical analyses. All statistical tests were 2 sided and a P value <0.05 was considered statistically significant.

Association analysis
To estimate the associations between plasma FGF23 and demographic and plasma and urinary parameters of calcium and phosphate metabolism, we conducted different mixed-effects linear regression models (Table 4, model groups 1–4). All models contain log-transformed plasma FGF23 as the dependent variable, fixed effects centered at zero, and family, center, and calendar day of blood sampling as random effects. If the quadratic term of a continuous predictor variable had a P value <0.1, it was kept in the model.

In model group 1, each model contains 1 predictor variable as an independent fixed effect, indicated in column 1. As an example, the first model 1 contains only the predictor variable age as a fixed effect, the second model 1 contains only the predictor variable sex as a fixed effect, and so forth. For model group 2, the first 4 predictor variables of age, sex, BMI, and eGFRcr in column 1 were included together as fixed effects in the same model, and the β coefficients of each predictor variable derived from this model are indicated in column 8 of Table 4. Therefore, in the first 2 lines of model group 2, the linear and quadratic term for age are adjusted for the other 3 predictor variables of sex, BMI, and eGFRcr, which then serve as covariables for age. Analogously, the predictor variable sex is adjusted for the other 3 predictor variables of age, BMI, and eGFRcr, which then serve as covariables for sex and so forth. The predictor variable plasma PTH was then added to this model, and therefore, in model group 2, plasma PTH is adjusted for the first 4 predictor variables of age, sex, BMI, and eGFRcr. The same applies for all other predictor variables listed in column 1 and described in model group 2.

Model group 3 was created as described for model group 2, but eGFRcr was replaced by eGFRcr-cys. Model group 4 was created as described for model group 2, but eGFRcr was replaced by 24-hour creatinine clearance.

All models were validated graphically for (i) homogeneity of variance by plotting residuals versus fitted values, (ii) normality of residuals by a quantile-quantile plot, (iii) highly influential observations by plotting the Cook’s distance for each data point. β coefficients and their 95% confidence intervals and P values of the predictor variables derived from valid models were described for linear and nonlinear terms.

DISCLOSURE
DF has served as a consultant for Otsuka Pharmaceuticals. DF has received unrestricted research funding from Novartis, Abbvie, and Otsuka Pharmaceuticals. All the other authors declared no competing interests.

ACKNOWLEDGMENTS
The SKIPOGH study is supported by the Swiss National Science Foundation (FN 33CM30-124087) and by intramural support of Lausanne, Geneva, and Bern University Hospitals. DF was supported by the Swiss National Centre of Competence in Research NCCR TransCure, the Swiss National Science Foundation (grants #31003A_135503 and #31003A_152829), and a Medical Research Position Award from the Foundation Prof. Dr. Max Cloëtta. DF and ND were supported by an unrestricted research grant from Abbvie and by intramural support of Bern University Hospital. Part of the results were presented at the Annual Meetings of the Swiss Society of Nephrology held December 5, 2014, in Interlaken, Switzerland, and December 3, 2015, in Basel, Switzerland.

SUPPLEMENTARY MATERIAL
Figure S1. Quadratic spline functions of the associations of eGFRcr (Creatinine Equation CKD-EPI 2009) with log transformed plasma FGF23 (A), with square root transformed plasma 1,25-dihydroxyvitamin D3 (B) and with log transformed plasma PTH (C). The shadowed areas represent 95% confidence intervals (CIs) for the fitted splines. The red dotted lines represent the highest eGFR at which the 95% CIs of the fitted splines do not overlap anymore.

Figure S2. Cubic spline functions of the associations of creatinine clearance (calculated from a timed urine collection and corrected for the BSA) with log transformed plasma FGF23 (A), with square root transformed plasma 1,25-dihydroxyvitamin D3 (B) and with log transformed plasma PTH (C). The shadowed areas represent 95% confidence intervals (CIs) for the fitted splines. The red dotted lines represent the highest eGFR at which the 95% CIs of the fitted splines do not overlap anymore.

Figure S3. Quadratic spline functions of the associations of eGFRcr-cys (Creatinine-Cystatin C Equation CKD-EPI 2012) with log transformed plasma FGF23 (A), with square root transformed plasma 1,25-dihydroxyvitamin D3 (B) and with log transformed plasma PTH (C) adjusted for the plasma 25-hydroxyvitamin D3 level. For adjustment, the effect of the plasma 25-hydroxyvitamin D3 level was held constant for all participants at a median value of 47.0 nmol/l in a corresponding linear regression model containing log transformed plasma FGF23 as dependent variable and a quadratic spline of eGFRcr-cys as independent variable with plasma 25-hydroxyvitamin D3 as a co-variable. The shadowed areas represent 95% confidence intervals (CIs) for the fitted splines. The red dotted lines represent the highest eGFR at which the 95% CIs of the fitted splines do not overlap anymore.

Table S1. Characteristics according to eGFRcr (Creatinine Equation CKD-EPI 2009) subgroups.

Table S2. Characteristics according to creatinine clearance (calculated from a timed urine collection and corrected for the BSA) subgroups.

Supplementary material is linked to the online version of the paper at www.kidney-international.org.

REFERENCES