Fibroblast growth factor-23 and mineral metabolism after unilateral nephrectomy

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Abstract

Background. Fibroblast growth factor -23 (FGF-23) is a key regulator of mineral metabolism. It regulates renal phosphate (Pi) reabsorption and calcitriol synthesis, and has an inhibitory effect on parathyroid hormone (PTH) secretion. FGF-23 increases early in chronic kidney disease (CKD), but the regulation of FGF-23 in mild-to-moderate renal dysfunction is not fully understood.

Methods. Nine healthy kidney donors underwent unilateral nephrectomy. Estimated glomerular filtration rate (eGFR) calculated from cystatin C and parameters of mineral metabolism: (Pi, ionized calcium, biointact PTH, intact FGF-23, calcitriol, and urinary excretion of calcium and Pi) were analysed before surgery, and one day, one week and three to six months after surgery.

Results. On the first post-operative day, PTH increased due to a decrease in the calcium level. One week after nephrectomy, the FGF-23 level increased from 31.8 ± 12.3 pg/mL to 55.8 ± 15.1 pg/mL, while PTH, Pi and calcium levels were unchanged compared to baseline. On follow-up, eGFR improved compared with its one-week value, and PTH and FGF-23 were unchanged compared to baseline. The calcitriol level decreased but was in the normal range at all points in time. The total amount of Pi in urine did not change, while the calcium excretion decreased significantly.

Conclusions. Pi homeostasis after nephrectomy is maintained by PTH on the first day. When serum calcium is stabilized and food intake resumed, FGF-23 rises, possibly in response to the Pi-load in relation to GFR.

Keywords: FGF23; FGF-23; mineral metabolism; nephrectomy; phosphate

Introduction

Phosphate (Pi) homeostasis is maintained by regulation of renal Pi reabsorption by the sodium-dependent phosphate co-transporters types a and c (NaPi-2a and NaPi-2c, respectively) in the proximal tubules. The phosphaturic hormones, parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF-23) decrease the reabsorption of filtered Pi by internalization and expression changes of these transporters [1,2].

FGF-23 is a bone-derived circulating peptide that plays a central role in the regulation of mineral metabolism. In addition to its phosphaturic properties, FGF-23 decreases calcitriol by downregulating the Cyp27b1 gene and upregulating Cyp24 [3]. FGF-23 also inhibits PTH secretion and synthesis [4,5].

The role of FGF-23 in chronic kidney disease (CKD) is a rapidly expanding area of research. Serum level of FGF-23 increases as glomerular filtration rate (GFR) declines [6,7]. FGF-23 levels are associated with several adverse outcomes in CKD, including faster progression rate [8], development of treatment-refractory secondary hyperparathyroidism [9] and increased mortality in haemodialysis patients [10]. The regulation of FGF-23 is not completely understood. FGF-23 synthesis is stimulated by calcitriol [11,12] and likely by hyperphosphataemia [13,14], although some studies indicate that it is rather the net phosphate balance and intestinal phosphate absorption that trigger FGF-23 production [15]. There is a correlation between PTH and FGF-23 in mild CKD independent of GFR or Pi levels [7]. The relative contribution of FGF-23, PTH and calcitriol in maintaining normal serum Pi levels in early CKD remains unexplored. Unilateral nephrectomy for kidney donation offers a possibility to examine the impact of a moderate reduction in GFR on mineral metabolism. To further elucidate the role of FGF-23 in regulation of mineral metabolism in early CKD, we evaluated temporal changes in serum FGF-23 in relation to mineral metabolism in healthy subjects before and after kidney donation.

Materials and methods

Subjects

Nine healthy kidney donors who underwent laparoscopic nephrectomy at Uppsala University Hospital were included in the study. They signed consent for participation in this study, which had been approved by the local
Main characteristics of the donors are given in Table 1, and the temporal variations in serum and urine biochemistries are presented in Table 2.

The serum level of Pi remained stable at all points in time, while ionized calcium decreased significantly, and PTH rose during the day of nephrectomy. Calcitriol decreased on the first day and remained significantly lower than at baseline. FGF-23 rose significantly at 1 week, but had returned to baseline on follow-up.

After 1 week, calcium and PTH had returned to baseline levels, and calcitriol level remained decreased, while the FGF-23 level had now increased to 55.8 ± 15.1 pg/mL (P < 0.01).

At follow-up, eGFR had recuperated, but it remained significantly lower than before the nephrectomy. Calcium, Pi, PTH and FGF-23 levels did not differ from preoperative levels, while the calcitriol level remained modestly decreased.

The total amount of phosphorus excreted in the urine and TmP/GFR was unchanged, while the TRP was lower than before nephrectomy. The calcium excretion had decreased from 6.1 ± 1.7 to 3.3 ± 1.4 mmol/day (P < 0.01).

Despite the fact that there were few study subjects, we did univariate linear correlation analysis between FGF-23 and PTH and the other parameters to get an indication of which correlations were strongest at different time points. Before nephrectomy, there were negative linear correlations between FGF-23 and Pi (r = −0.75, P = 0.02) and between FGF-23 and calcitriol (r = −0.86, P = 0.003). The correlation between FGF-23 and TmP/GFR was borderline significant in this small sample (r = 0.65, P = 0.06).

Calcitriol correlated with Pi (r = 0.7, P = 0.04) and FGF-23 but not with calcium or PTH.

### Table 1. Characteristics of donors

<table>
<thead>
<tr>
<th></th>
<th>n = 9</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>3/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.7 ± 9.3</td>
<td>38–69</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.4 ± 11.6</td>
<td>53–86</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2 ± 2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>systolic</td>
<td>125 ± 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>diastolic</td>
<td>82 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>67.7 ± 8.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td></td>
<td>1.45</td>
<td>ND</td>
</tr>
</tbody>
</table>

BMI, body mass index [weight (kg) / length (m)²].

### Table 2. Parameters of mineral metabolism before and after nephrectomy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal range</th>
<th>Before nephrectomy</th>
<th>After 1 day</th>
<th>After 1 week</th>
<th>After 3–6 months</th>
<th>ANOVA for repeated measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>&gt;90</td>
<td>106.6 ± 17.2</td>
<td><strong>76.2 ± 23.5</strong></td>
<td><strong>54.2 ± 12.8</strong></td>
<td><strong>69.9 ± 13.7</strong></td>
<td>P = 0.0001</td>
</tr>
<tr>
<td>Phosphorus (mmol/L)</td>
<td>0.75–1.40</td>
<td>1.19 ± 0.20</td>
<td>1.07 ± 0.20</td>
<td>1.21 ± 0.10</td>
<td>1.04 ± 0.16</td>
<td>P = 0.14</td>
</tr>
<tr>
<td>Calcium, ionized (mmol/L)</td>
<td>1.10–1.30</td>
<td>1.22 ± 0.04</td>
<td><strong>1.12 ± 0.05</strong></td>
<td>1.23 ± 0.04</td>
<td>1.23 ± 0.03</td>
<td>P = 0.0001</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>6.6–38.4</td>
<td>20.0 ± 7.4</td>
<td><strong>28.0 ± 12.5</strong></td>
<td>14.5 ± 7.5</td>
<td>18.7 ± 7.6</td>
<td>P = 0.0002</td>
</tr>
<tr>
<td>FGF-23 (ng/mL)</td>
<td>10–50</td>
<td>31.8 ± 12.3</td>
<td><strong>55.8 ± 15.1</strong></td>
<td>36.3 ± 13.7</td>
<td>P = 0.002</td>
<td></td>
</tr>
<tr>
<td>Calcitriol (pmol/L)</td>
<td>40–130</td>
<td>129.3 ± 44.7</td>
<td><strong>87.3 ± 25.1</strong></td>
<td>86.7 ± 26.5</td>
<td><strong>102.0 ± 38.2</strong></td>
<td>P = 0.0073</td>
</tr>
<tr>
<td>dU-P (mg/m² 24 h)</td>
<td>≤38</td>
<td>28.0 ± 6.5</td>
<td>24.8 ± 7.3</td>
<td>ND</td>
<td>30.7 ± 9.9</td>
<td>P = 0.4</td>
</tr>
<tr>
<td>FE-Pi (%)</td>
<td>5–20</td>
<td>15.2 ± 8.8</td>
<td><strong>19.1 ± 13</strong></td>
<td>ND</td>
<td><strong>27 ± 17.6</strong></td>
<td>P = 0.002</td>
</tr>
<tr>
<td>dU-Ca (mmol/24 h)</td>
<td>2.8–8.0</td>
<td>6.1 ± 1.7</td>
<td><strong>4.6 ± 2.1</strong></td>
<td>ND</td>
<td><strong>3.3 ± 1.4</strong></td>
<td>P = 0.001</td>
</tr>
<tr>
<td>TmPGFR</td>
<td>0.7–1.45</td>
<td>1.04 ± 0.23</td>
<td>0.89 ± 0.20</td>
<td>ND</td>
<td>0.71 ± 0.12</td>
<td>P = 0.07</td>
</tr>
</tbody>
</table>

ANOVA for repeated measurements and post hoc analysis were performed. eGFR, estimated glomerular filtration rate; FE-Pi, fractional excretion of Pi were calculated as [urine Pi (mmol/24 h) × serum creatinine (mmol/L) × 100] / [urine Pi (mmol/L) × serum creatinine (mmol/24 h)]. TmPGFR, tubular maximum phosphate reabsorption per glomerular filtration rate, derived from the nomogram by Walton and Bijovet.

*P < 0.05

**P < 0.01 for differences from “before nephrectomy”.

The technique used for the laparoscopic nephrectomy has been described elsewhere [16]. The donors received ~6 L of intravenous fluid and a small dose of furosemide, during the first 24 h postoperatively, to maintain a brisk diuresis. No complications related to the surgery or postoperative care occurred, and all could be discharged from the hospital within 1 week.

**P < 0.01 for differences from

Used for all statistical analysis.

The correlation between FGF-23 and TmP/GFR was borderline significant in this small sample (r = 0.65, P = 0.06).

Calcitriol correlated with Pi (r = 0.7, P = 0.04) and FGF-23 but not with calcium or PTH.

The total amount of phosphorus excreted in the urine and TmP/GFR was unchanged, while the TRP was lower than before nephrectomy. The calcium excretion had decreased from 6.1 ± 1.7 to 3.3 ± 1.4 mmol/day (P < 0.01).

Despite the fact that there were few study subjects, we did univariate linear correlation analysis between FGF-23 and PTH and the other parameters to get an indication of which correlations were strongest at different time points. Before nephrectomy, there were negative linear correlations between FGF-23 and Pi (r = −0.75, P = 0.02) and between FGF-23 and calcitriol (r = −0.86, P = 0.003). The correlation between FGF-23 and TmP/GFR was borderline significant in this small sample (r = 0.65, P = 0.06).

Calcitriol correlated with Pi (r = 0.7, P = 0.04) and FGF-23 but not with calcium or PTH.

**P = 0.0073
After 1 week, FGF-23 did not correlate with any of the other parameters, while there was a negative correlation between calcitriol and PTH \((r = -0.89, P = 0.02)\). On follow-up after 3–6 months, FGF-23 and PTH correlated \((r = 0.76, P = 0.05)\).

**Discussion**

In the current study, we evaluated temporal variations in serum FGF-23 and other parameters of mineral metabolism, related to a reduction in GFR, in healthy kidney donors. We found a temporary rise in FGF-23 at 1 week after nephrectomy and a moderate decrease in calcitriol level from the first postoperative day.

On the first postoperative day, after fasting, nephrectomy and forced diuresis, there was a tendency towards hypocalcaemia and an appropriate rise in PTH. The calcitriol level declines significantly, but it is not halved like the renal mass, implicating that calcitriol synthesis in the remaining kidney is stimulated. There is no change in the FGF-23 level at this point in time. The renal excretion of calcium decreases significantly, compared with the day before surgery, while the total amount of urinary Pi remained unchanged. Since there was no oral intake of calcium or phosphate that day, we assume that PTH also induced the utilization of bone mineral as a source of calcium and phosphorus. The phosphaturic effect of PTH may be more relevant than that of FGF-23 in the immediate response to reduced renal function. We also conclude that the decrease in GFR does not cause a passive increase in FGF-23.

After 1 week, there is a significant rise in FGF-23. The reasons for this rise could be an increased Pi load on the kidney, a delayed effect of the initial increase in PTH secretion or accumulation due to decreased renal clearance.

We speculate that the decrease in the renal filtration of Pi induces a change in the fluctuations of Pi and calcium flow between the gastrointestinal tract, bone and kidney that can be sensed by the osteocytes. This mechanism does not become fully active until the serum calcium level and oral intake of Pi and calcium have normalized. Alternatively, the rise in PTH level, which occurred before that of FGF-23, could indicate that PTH directly stimulates FGF-23 synthesis. Indeed, recent in vitro data support PTH as a stimulator of FGF-23 in bone cells [18].

Finally, decreased renal clearance of FGF-23 may contribute to its accumulation, although this appears less likely given that we measured whole FGF-23, with a short elimination half-life of 30–60 mins [17], and the most important method of elimination is enzymatic cleavage.

On follow-up, when eGFR has improved, PTH and FGF-23 have returned to baseline levels. The calcitriol remains lower than at baseline, but is still in the normal range, and the urinary excretion of calcium is significantly decreased. Even in this small group of subjects, there is a significant positive correlation between FGF-23 and PTH on follow-up, in accordance with earlier observations in mild-to-moderate CKD [7]. Nevertheless, the decrease in TRP% is caused by intrinsic mechanisms in the kidney rather than by the hormonal effects of PTH or FGF-23.

We conclude that FGF-23 and PTH fine-tune the calcitriol level to be optimal for the actual GFR and net Pi balance, and the gastrointestinal Pi uptake may signal the calcitriol level, in relation to GFR, to the osteocytes.

Limitations of this study are its small size and lack of control of phosphorus intake. Also, we did not measure possible changes in calcidiol levels, but given that the donors had normal PTH and calcitriol levels before nephrectomy and the long plasma half-life of calcidiol, there should not be any significant deficiency during the study. Finally, the generalizability to early CKD is limited since there were several factors affecting mineral metabolism close to surgery and the GFR improved on follow-up.

The strength of this interventional study is that it examines healthy individuals undergoing a controlled and rapid reduction in GFR, using robust and biologically relevant chemical analyses. Despite the finding that the mineral metabolism was within the normal range and the fact that there are several possible explanations for the rise in FGF-23 level, our study supports the idea that FGF-23 modulates mineral metabolism, secondary to PTH.

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**Conflict of interest statement.** Ö.L. has received lecture fees from Aagen and Abbott; T.E.L. has received honoraria/lecture fees from Genzyme, Abbott, Aagen and Swedish Orphan; and T.L. has received lecture fees from Aagen. The other authors have nothing to declare. The results in this study have not been published before, except in abstract form.

**References**

Glomerular and tubulointerstitial infiltrates in chronic allograft dysfunction


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Immunohistochemical characterization of glomerular and tubulointerstitial infiltrates in renal transplant patients with chronic allograft dysfunction

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*Drs Divella and Rossini equally contributed to the study.

Abstract

**Background.** The term chronic allograft nephropathy (CAN) was deleted in the Eighth Banff Classification and two new categories were introduced: chronic T-cell-mediated rejection (CTMR) and chronic active humoral rejection (CAHR). The aim of this study was to revise our CAN cases diagnosed in the last 4 years, analyse allograft survival rates and identify types of infiltrating cells in the different settings.

**Methods.** Seventy-nine patients with biopsy-proven CAN were examined and classified into four groups according the Banff 2005 criteria: CTMR, CAHR, chronic calcineurin inhibitor toxicity (CNITOX) and interstitial fibrosis and tubular atrophy not otherwise specified (NOS). CD4, CD8, CD20, CD68, CD103, Foxp3 and IL-17 protein expression and C4d deposits were investigated.

**Results.** We diagnosed 20 CTMR, 13 CAHR, 28 CNITOX, and 18 NOS. Death-censored graft survival at 4 years from renal biopsy was worse in CAHR compared with the other types of chronic injury. Glomerular CD8+ cells were increased in CTMR vs CNITOX and NOS. Interstitial CD4+ and CD8+ cells were increased in CTMR vs CNITOX. CD68+ cells in glomerular and peritubular capillaries were higher in CAHR vs CNITOX, CTMR and NOS. CD103+ cells were higher in cases with tubulitis than in those without. T regulatory and T helper 17 cells were rarely observed in the different settings.

**Conclusions.** Graft survival was worse in patients with CAHR. The presence of any grade transplant glomerulopathy and chronic allograft vasculopathy are poorer prognostic factors. Infiltrating CD8+, CD103+ and CD4+ cells may help to differentiate CTMR from other types of chronic injury, thus improving diagnostic/prognostic features of biopsy in patients with chronic allograft dysfunction.

**Keywords:** Banff schema; chronic active antibody-mediated rejection; chronic T-cell-mediated rejection; immunohistochemical markers; renal transplantation

Introduction

‘Chronic allograft nephropathy’ (CAN) represents the leading cause of late allograft loss in kidney transplant patients. However, the term ‘CAN’ does not indicate a specif-