Chapter 9

High Potassium Intake Reduces Fibroblast Growth Factor 23 to Increase Renal Phosphate Reabsorption

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In Preparation (Embargo).
Abstract

Background: High plasma concentrations of the phosphate-regulating hormone fibroblast growth factor 23 (FGF-23) are robustly associated with cardiovascular morbidity and mortality. The determinants of FGF-23 concentration are not completely understood, hampering development of strategies to reduce FGF-23. Since dietary potassium increases serum phosphate, we hypothesized that potassium supplementation would reduce FGF-23 levels.

Methods: Post hoc analysis of a randomized, blinded, placebo-controlled cross-over trial in which 36 untreated pre-hypertensives underwent three study periods of four weeks on a fully controlled, on-site diet combined with capsules containing 3 grams of potassium/day, 3 grams of sodium/day or placebo, respectively, in random order. Dietary potassium intake was kept constant at 2.3 grams/d. Blood and 24h-urine were collected after each study period. Plasma C-terminal FGF-23 was measured by ELISA, parathyroid hormone (PTH) by radioimmunoassay. Outcomes at the end of each supplementation period were compared with placebo using linear mixed models.

Results: Participants (67% male) were 66.0 ± 9.3 (mean ± SD) years old and had an eGFR (cystatin C-based CKD-EPI) of 84 ± 13 mL/min/1.73m², that was not influenced by potassium supplementation (P=0.6). Potassium supplementation increased urinary potassium excretion from 55.3 ± 16.7 to 118.1 ± 32.2 mmol/d (P<0.001). Serum phosphate increased from 1.10 ± 0.19 to 1.15 ± 0.18 mmol/L (P=0.004), in line with an increase in TmP/GFR from 0.93 ± 0.21 to 1.01 ± 0.20 mmol/L (P<0.001). Plasma FGF-23 decreased from 114.3 RU/mL (geometric mean; 95% CI, 96.2 to 135.8) to 108.5 RU/mL (95% CI, 93.0 to 126.6; P=0.01), without changes in PTH and 25(OH)-vitamin D₃. Excretion of sodium, phosphate and urea did not materially change.

Conclusions: Dietary potassium supplementation induced a reduction in FGF-23, an increased TmP/GFR, the tubular set point for phosphate reabsorption, and a higher serum phosphate level. This effect was independent of PTH or 25(OH)-vitamin D₃. Sodium supplementation lowered FGF-23 and increased serum phosphate. Potassium supplementation may be a novel strategy to reduce FGF-23 levels and subsequently improve adverse outcomes in chronic kidney disease and heart failure.
**Introduction**

Fibroblast growth factor 23 (FGF-23) has been recognized as a key regulator of phosphate homeostasis. FGF-23 stimulates renal phosphate excretion, and inhibits the production of parathyroid hormone (PTH) and active, 1,25-dihydroxy vitamin D (1). A higher concentration of FGF-23 is associated with higher mortality risk, particularly in high-risk populations as haemodialysis patients (2, 3) and predialysis chronic kidney disease (4, 5), but also in the general population (6, 7). However, our understanding of determinants and regulators of FGF-23 is incomplete, and this hampers efforts to reduce FGF-23. Serum phosphate concentrations and renal function are tightly correlated with FGF-23 concentrations, but strategies that reduce serum phosphate levels are at best modestly effective in lowering FGF-23 (8, 9). Possibly, dietary factors contribute to FGF-23 physiology.

A recent study demonstrated that the Western diet is associated with higher circulating FGF-23 levels (10). Besides being rich in phosphate, the Western diet is also relatively poor in potassium content. Lower potassium intakes are associated with higher risk of cardiovascular events (11), end stage renal disease (12), and mortality (13). Interestingly, the observational study found an inverse association between potassium intake and FGF-23 levels (10). Studies from the 1980s have demonstrated that potassium supplementation increases serum phosphate concentrations in healthy volunteers independent of PTH (14). In preclinical studies, potassium increased tubular reabsorption of phosphate (15). The mechanism of this effect of potassium on phosphate is unknown. We hypothesize an inhibiting effect of potassium on FGF-23 concentrations as a candidate mechanism. If so, potassium supplementation will lower FGF-23 as a mechanism that increases phosphate concentration. To test this hypothesis we measured phosphate and FGF-23 in a post hoc analysis of a randomized, full-dietary, placebo-controlled trial of potassium supplementation.

**Methods**

**Participants**

We analyzed a double-blinded, randomized, placebo-controlled, crossover study that assessed the effects of potassium and sodium supplementation on blood pressure. The protocol has extensively been described (16). Eligible participants were 40–80 years old, with a fasting office systolic blood pressure of 130–159 mmHg. Exclusion criteria were diabetes mellitus, renal diseases including chronic kidney disease, gastrointestinal and liver diseases. Participants were also ineligible for participation if they were current smokers; had a BMI > 40 kg/m²; used medication that affects cardiovascular system; nutritional supplements; were on a energy-restricted
or medically prescribed diet; had unstable weight or excessive alcohol use. Participants were recruited from December 2011 to April 2012.

**Study Design**

Eligible participants were on a fully-controlled diet according to individual energy needs. The research facility supplied 90% of the daily energy needs, the remaining 10% were chosen by the participants from a list of products that were low in sodium and potassium. A 2500 kcal-diet provided 2.3 grams of potassium, 2.4 grams of sodium and 82.2 grams of protein. After a run-in period of 1 week on the diet, participants underwent three 4-week treatment periods. The treatment periods consisted of the daily use of 8 capsules (Microz, Geleen, the Netherlands), that contained in total 3 grams of potassium, 3 grams of sodium (7.5 grams of salt) or placebo, in randomized order.

**Measurements**

Participants underwent venous blood sampling after each treatment period at fixed time points of the day throughout the study, and handed in a 24-h urinary collection. Serum, EDTA-plasma and urine samples were stored at –80°C, and electrolytes were assessed by routine laboratory procedures (Modular P, Roche Diagnostics, Mannheim, Germany). C-terminal FGF-23 was determined in EDTA-plasma by enzyme-linked immunosorbent assay (ELISA, Immutopics, San Clemente, CA, USA). Parathyroid hormone (PTH) was measured by radioimmunoassay, and 25-hydroxy vitamin D₃ (25[OH]-vitamin D₃) with isotope dilution–online solid phase extraction liquid chromatography–tandem mass spectrometry, both in EDTA-plasma. The tubular maximum reabsorption / GFR (TmP/GFR) was calculated as a measure of the phosphate threshold using the next formula (17):

\[
TmP/GFR = \text{serum phosphate (mmol/L)} - (\text{urinary phosphate [mmol/L] × serum creatinine [mmol/L]}) / \text{urinary creatinine [mmol/L]}
\]

**Statistical Analysis**

Normally-distributed data are presented as means ± standard deviation, whereas data that did not follow the normal distribution are presented as geometric mean and 95% confidence interval. For each outcome measure, we used a mixed-effects model with covariance structure compound symmetry to estimate the effect of active treatment compared with placebo. Fixed effects were ‘treatment’ and ‘period’, random effect was participant number. Variables were natural log (Ln) transformed when appropriate, as assessed with histograms and Q-Q plots. Analysis were performed in SAS 9.3 (SAS Institute, Cary, North Carolina, USA).
Table 1. Effects of Potassium or Sodium Supplementation on Mineral Bone Metabolism Parameters

<table>
<thead>
<tr>
<th>Serum</th>
<th>Mean ± SD</th>
<th>Mean difference (95%)</th>
<th>Potassium vs Placebo</th>
<th>P-value</th>
<th>Sodium vs Placebo</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum phosphate, mmol/L</td>
<td>1.15 ± 0.19</td>
<td>1.06 ± 0.21</td>
<td>0.05 (0.02 to 0.09)</td>
<td>0.004</td>
<td>−0.04 (−0.08 to 0.00)</td>
<td>0.03</td>
</tr>
<tr>
<td>Serum calcium, mmol/L</td>
<td>2.34 ± 0.08</td>
<td>2.33 ± 0.08</td>
<td>−0.01 (−0.03 to 0.02)</td>
<td>0.6</td>
<td>−0.01 (−0.04 to 0.01)</td>
<td>0.2</td>
</tr>
<tr>
<td>FGF-23, RU/mL(a)</td>
<td>108.5 (93.0 to 126.6)</td>
<td>108.7 (91.7 to 128.8)</td>
<td>−0.05 (−0.09 to −0.01)</td>
<td>0.01</td>
<td>−0.05 (−0.09 to −0.01)</td>
<td>0.02</td>
</tr>
<tr>
<td>PTH, pmol/L(a)</td>
<td>4.36 (3.84 to 4.94)</td>
<td>4.37 (3.93 to 4.85)</td>
<td>0.00 (−0.07 to 0.06)</td>
<td>0.9</td>
<td>0.00 (−0.06 to 0.07)</td>
<td>0.9</td>
</tr>
<tr>
<td>25(OH)-vitamin D3(b)</td>
<td>59.0 ± 19.0</td>
<td>58.3 ± 18.1</td>
<td>0.9 (−1.6 to 3.3)</td>
<td>0.5</td>
<td>−0.8 (−3.3 to 1.7)</td>
<td>0.5</td>
</tr>
<tr>
<td>Urine</td>
<td>Sodium excretion, mmol/24h</td>
<td>96.5 ± 39.0</td>
<td>202.9 ± 54.8</td>
<td>−8.9 (−25.4 to 7.6)</td>
<td>0.3</td>
<td>97.6 (81.0 to 114.1)</td>
</tr>
<tr>
<td>Potassium excretion, mmol/24h</td>
<td>118.1 ± 32.2</td>
<td>53.2 ± 16.6</td>
<td>62.9 (54.9 to 70.8)</td>
<td>&lt;0.001</td>
<td>−2.2 (−10.2 to 5.7)</td>
<td>0.6</td>
</tr>
<tr>
<td>Phosphate excretion, mmol/24h</td>
<td>24.36 ± 9.58</td>
<td>24.45 ± 7.27</td>
<td>−0.24 (−0.69 to 0.21)</td>
<td>0.3</td>
<td>1.16 (0.70 to 1.61)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium excretion, mmol/24h</td>
<td>4.05 ± 2.15</td>
<td>5.45 ± 2.51</td>
<td>4.5 (−2.47 to 2.43)</td>
<td>0.99</td>
<td>0.05 (−2.40 to 2.50)</td>
<td>0.98</td>
</tr>
<tr>
<td>Urea excretion, mmol/24h</td>
<td>372 ± 103</td>
<td>363 ± 120</td>
<td>16 (−11 to 43)</td>
<td>0.2</td>
<td>7 (−20 to 34)</td>
<td>0.6</td>
</tr>
<tr>
<td>TmP/GFR</td>
<td>1.01 ± 0.20</td>
<td>0.91 ± 0.22</td>
<td>0.07 (0.03 to 0.11)</td>
<td>&lt;0.001</td>
<td>−0.02 (−0.06 to 0.01)</td>
<td>0.2</td>
</tr>
<tr>
<td>Fractional excretion of phosphate, %</td>
<td>13.3 ± 4.2</td>
<td>14.7 ± 4.5</td>
<td>−2.50 (−3.75 to −1.25)</td>
<td>&lt;0.001</td>
<td>−1.01 (−2.26 to 0.24)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

a) Values are geometric mean and 95% CI for FGF-23 and PTH, differences are change in Ln-transformed variable. b) Due to three missing samples, analysis performed for potassium on n=36 (no missing data), placebo n=35, sodium n=34, respectively. Abbreviations: SD, standard deviation; FGF-23, fibroblast growth factor 23; PTH, parathyroid hormone; TmP/GFR, tubular maximum reabsorption of phosphate per glomerular filtration rate.
Results

Population Characteristics
The 36 participants were 65.8 years old (range 47–80), predominantly male (67%) with a body mass index of 27.2 ± 4.6 kg/m². Participants had slightly elevated blood pressure at screening (average 145/81 mmHg). Baseline characteristics have been reported previously (16, 18).

Effect of Potassium Supplementation
Potassium supplementation increased potassium excretion by 62.9 mmol (Table 1). This increase of 62.8 mmol (2459 mg) is in accordance with ~80% gastrointestinal uptake of the 3 grams supplemented potassium. Serum phosphate concentration increased from 1.10 ± 0.19 to 1.15 ± 0.19 mmol/L (P= 0.004), whereas serum calcium concentration was unchanged. The increase in serum phosphorus was paralleled by an increase in phosphate tubular reabsorption, as assessed by TmP/GFR, from 0.93 ± 0.21 to 1.01 ± 0.20. Urinary phosphate excretion did not change (Figure 1, panels A–C). We analyzed three hormones that are involved in renal phosphate handling. Potassium supplementation reduced FGF-23 concentrations from 114.3 RU/mL (geometric mean; 95% CI, 96.2 to 135.8) to 108.5 RU/mL (95% CI, 93.0 to 126.6; P=0.01). Potassium supplementation did not influence 25(OH)-vitamin D₃ or PTH concentrations (Figure 1, panels D–F). Potassium supplementation did not change sodium or urea excretion.

Effects of Sodium Supplementation
Sodium supplementation increased 24-h urinary sodium excretion by 97.6 mmol/day, whereas potassium excretion did not change (P=0.6). Serum phosphate levels were a bit lower, and surprisingly, FGF-23 concentrations were lower as well (Table 2). Here, TmP/GFR did not change (P=0.1), neither did 25(OH)-vitamin D₃, PTH nor 24-hour phosphate excretion.

Discussion

In this randomized, placebo- and fully dietary controlled trial potassium supplementation reduced FGF-23 concentrations, with a concomitant rise of renal phosphate reabsorption and serum phosphate levels, without an effect on PTH or 25(OH)-vitamin D₃. These data suggest that potassium supplementation may be a novel strategy to reduce FGF-23 levels in prehypertensives.

A stimulatory effect of potassium on phosphate reabsorption has been described in rats already in 1983 (15). At variance with the animal study, PTH did not change in our study and is therefore unlikely to explain the observed effect on serum phosphate. Our results are in line with a previous study in healthy human volunteers, where potassium supplementation increased
serum phosphate levels (14). Although our patients were older and prehypertensive, they were otherwise healthy and the mean increase of 0.05 mmol/L in serum phosphate is comparable with the 0.07 mmol/L increase Sebastian et al. reported (14). The same study underscored the specificity of potassium, because the effect was present regardless whether potassium bicar-
bonate or potassium chloride was used. In line, epidemiological studies report that a population living in Ghana had a diet more rich in vegetables (and thus potassium), accompanied by higher serum phosphate, lower fractional excretion of phosphate, and lower FGF-23 concentrations compared with a population of African ancestry that lived in the United States (19). This mirrors our results of potassium treatment, although potassium intake was not reported. To summarize our findings, we propose that potassium supplementation lowers FGF-23 to increase TmP/GFR, and thus renal phosphate reabsorption. This temporarily reduces phosphate excretion, until a new steady state with higher serum phosphate concentration has been reached (Figure 2).

![Figure 2. Schematic representation of our hypothesis. Potassium supplementation starts at day 0. This results in a drop of FGF-23, and thus increases the renal set point for phosphate reabsorption, TmP/GFR. Phosphate excretion temporarily falls, and reaches a new steady state at a higher serum phosphate concentration. Question marks depict uncertainty about the time course of changes in FGF-23 and PTH, as measurements took place only after 30 days.](image)

The increase in serum phosphate may be disconcerting, as frank hyperphosphataemia (>1.78 mmol/L) is an established risk factor for mortality in haemodialysis patients (20). Even more, serum phosphate concentrations >1.13 mmol/L were associated with increased mortality risk in patients with CKD (21). However, whether a potassium-induced rise of serum phosphate levels is also detrimental cannot be derived from these data.

We also evaluated the effect of sodium supplementation on FGF-23. Although sodium lowered FGF-23 concentrations, this effect was not accompanied by changes in TmP/GFR, and occurred together with a counterintuitive reduction of serum phosphate concentrations. This may be explained by sodium-induced calcium excretion, which was inversely correlated with FGF-23 concentrations in previous studies (22), and low serum calcium levels correlate positively with low FGF-23 levels (23). In line, the calcium × phosphate product is a stronger correlate of FGF-23 concentrations than when phosphate or calcium are considered individually (24). Although serum calcium levels did not change in our study, it is possible that increased calciuria contributed to a tendency for a lower calcium × phosphate product, which may have elicited the reduction in FGF-23 concentrations.
The mechanism how a potassium load reduces FGF-23 is unknown. A possible explanation for this interaction may lie in the effects of potassium load on the renal tubuli. A potassium load inhibits the sodium (Na⁺) chloride cotransporter (NCC) by rapid dephosphorylation in mice (25), regardless whether potassium is administrated orally (25) or intravenously (26). This NCC dephosphorylation also occurs when potassium is supplemented to a low sodium diet (27), this mirrors our study design. Putatively, this dephosphorylation interacts with upstream WNK/SPAK signaling, which has been implied to lower FGF-23 concentrations (28). Alternatively, potassium may directly reduce the formation of FGF-23 in the osteocytes in the bone. The extracellular matrix in the bone has fivefold higher potassium concentration compared with extracellular fluid, a gradient that is maintained by active transport mechanisms (29). We postulate that the bone may serve as a buffer for potassium load. Possibly, the osteocytes are sensitive to increases in potassium load and respond by reducing the production of FGF-23.

Part of the beneficial effects of high potassium intake may thus be mediated by potassium-induced reduction of FGF-23 concentrations, and the ensuing putative beneficial effects on volume status (18) may overcome the possible adverse effects of a higher serum phosphate level. This explains why a diet rich in potassium and low FGF-23 concentrations are both robustly associated with better cardiovascular outcomes. For potassium this is traditionally explained by its blood pressure-lowering effects. A meta-analysis found that for a mean increase of 51 mmol potassium per day, blood pressure was reduced by 3.3/2.1 mmHg (30). Potassium supplementation also lowered blood pressure in our cohort (16). Interestingly, an interaction between volume status and FGF-23 has been demonstrated in experimental studies where FGF-23 increases sodium retention and blood pressure by upregulation of NCC (32), further corroborating that potassium and FGF-23 may be related by their effects on volume status. So, the drop of FGF-23 reduces the putative stimulatory effect of FGF-23 on NCC (32), thus facilitating sodium-potassium exchange in order to maintain potassium balance, at the expense of an increase in serum phosphate concentrations.

Reduction of FGF-23 is of paramount importance, given its strong association with increased mortality across several populations (2-7). Several lines of evidence suggest that FGF-23 itself is involved in increased cardiovascular morbidity and mortality as an effector and not merely a correlate. Two different hypotheses exist to explain the detrimental effect of FGF-23 on the cardiovascular system. First, FGF-23 may directly promote left ventricular hypertrophy (33), possibly by stimulation of the FGF4 receptor (34). The more recent hypothesis suggests that FGF-23 may induce volume retention (32). In line, FGF-23 may increase risk of heart failure (5, 35). Our results indicate a novel and readily targetable pathway that can reduce FGF-23 concentrations.

Strengths of this study include the fully-controlled diet and the use of potassium, sodium and placebo capsules. The crossover design increased power, as every participant served as his own
control. Weaknesses of this study are the limited sample size and that measurements were only performed at the end of each diet period. The exact time at which reduction of FGF-23 occurred is therefore unknown, and conclusions whether the reduction persists over time cannot be drawn. This is not of trivial importance, as phosphate supplementation temporarily increased FGF-23 levels after four weeks, only to return to baseline values after eight weeks of supplementation (36) —without affecting plasma phosphate levels. Obviously, such studies do not exist for potassium at this time. Finally, our study does not allow to identify the mechanism of the effect of potassium supplementation on FGF-23 levels.

In conclusion, we demonstrated in a fully dietary and placebo-controlled study that potassium supplementation increases phosphate retention and reduces FGF-23. Our study suggests that FGF-23 connects potassium and phosphate homeostasis; this may explain why a lifestyle rich in potassium may yield beneficial cardiovascular and renal outcomes.

Acknowledgements.

The authors acknowledge W.A. Dam and B.M. Aarts for technical assistance. The original research was supported by research grant CH001 from TI Food and Nutrition, a public-private partnership on precompetitive research in food and nutrition. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
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