Response of fibroblast growth factor 23 to volume interventions in arterial hypertension and diabetic nephropathy

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Abstract

Fibroblast growth factor 23 (FGF-23) rises progressively in chronic kidney disease and is associated with adverse cardiovascular outcomes. FGF-23 putatively induces volume retention by upregulating the sodium-chloride cotransporter (NCC). We studied whether, conversely, interventions in volume status affect FGF-23 concentrations.

We performed a post hoc analysis of 1) a prospective saline infusion study with 12 patients with arterial hypertension who received 2L of isotonic saline over 4 hours, and 2) a randomized controlled trial with 45 diabetic nephropathy (DN) patients on background angiotensin-converting enzyme-inhibition (ACEi), who underwent 4 6-week treatment periods with add-on hydrochlorothiazide (HCT) or placebo, combined with regular sodium (RS) or low sodium (LS) diet in a cross-over design. Plasma C-terminal FGF-23 was measured by ELISA (Immuno) after each treatment period in DN and before and after saline infusion in hypertensives.

The patients with arterial hypertension were 45 ± 13 (mean ± SD) years old with an estimated glomerular filtration rate (eGFR) of 101 ± 18 mL/min/1.73m². Isotonic saline infusion did not affect FGF-23 (before infusion: 68 median [first to third quartile: 58–97] relative unit (RU)/mL, after infusion: 67 [57–77] RU/mL, P = 0.37). DN patients were 65 ± 9 years old. During ACEi + RS treatment, eGFR was 65 ± 25 mL/min/1.73 m² and albuminuria 649 mg/d (230–2008 mg/d). FGF23 level was 94 (73–141) RU/mL during ACEI therapy. FGF-23 did not change significantly by add-on HCT (99 [74–148] RU/mL), LS diet (99 [75–135] RU/mL), or their combination (111 [91–160] RU/mL), P = 0.15.

Acute and chronic changes in volume status did not materially change FGF-23 in hypertensive patients and DN, respectively. Our data do not support a direct feedback loop between volume status and FGF-23 in hypertension or DN.

Abbreviations: eGFR = estimated glomerular filtration rate, CKD = chronic kidney disease, CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration group, DN = diabetic nephropathy, FGF-23 = fibroblast growth factor 23, HCT = hydrochlorothiazide, In = natural logarithm, LS = low sodium, NCC = sodium-chloride cotransporter, RAAS = renin-angiotensin-aldosterone system, RS = regular sodium, RU = relative unit.

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1. Introduction

The phosphaturic hormone fibroblast growth factor 23 (FGF-23) is a central regulator of calcium-phosphate metabolism. In moderate-to-severe chronic kidney disease (CKD), FGF-23 concentrations progressively increase in an attempt to keep phosphate balance.[1] More recently, a higher concentration of FGF-23 in CKD patients has been linked with an increased risk of cardiovascular events, particularly with decompensated heart failure, which in CKD is often caused by hypervolemia (as reviewed in Ref. [3]). Similar associations between plasma FGF-23 concentrations and the incidence of decompensated heart failure were found among individuals without overt CKD.[4-5]

In line, animal experiments suggest that FGF-23 may directly induce left ventricular hypertrophy via the calcineurin-NFAT pathway.[6,7] Yet, the role of FGF-23 in cardiovascular disease in patients without advanced CKD is subject of debate,[8,9] and other pathophysiological pathways may be involved.[10] Notably, in addition to the proposed direct effects of FGF-23 on the myocardium, volume overload could contribute to the association between increased FGF-23 and decompensated heart failure observed in cohort studies. This hypothesis is fueled by recent experimental data, which suggest that FGF-23 may activate the sodium-chloride cotransporter (NCC) in the distal tubule, inducing volume expansion, hypertension, and subsequently left ventricular hypertrophy.[11] The diuretic hydrochlorothiazide (HCT), which inhibits the NCC in the distal tubule, prevented these presumed off-target effects. These observations add to previously documented independent associations between FGF-23 and markers of volume status,[12] and could at least partly explain the previously observed association between a higher FGF-23 level and an impaired antiproteinuric response to volume depletion.[13] Conversely, recent data suggest that changes in volume status may modulate FGF-23 concentrations at least in hemodialysis.[14] This suggests the presence of a feedback loop, where FGF-23 increases volume load, and an increase in volume may reduce FGF-23. Vice versa, a reduction in volume load may in turn increase FGF-23. In the present study, we investigated the effect of interventions in volume status on FGF-23 in 2 independent settings. We first analyzed the acute effects of intravenous volume loading on plasma FGF-23 in 12 hypertensive individuals without overt CKD. Second, we investigated the chronic impact of dietary sodium restriction and HCT therapy on plasma FGF-23 in patients with diabetic CKD during standardized angiotensin-converting enzyme inhibition (ACEi). To expand knowledge on the hypothesized bidirectional relationship between FGF-23 and volume regulation, we analyzed among the same patients the extent by which FGF-23 plasma concentrations predict the antiproteinuric response to dietary sodium restriction and HCT therapy.

2. Materials and methods

2.1. Study design and measurements

To investigate the effects of acute volume load on FGF-23 plasma concentrations, we analyzed 12 outpatients with arterial hypertension but without overt CKD (defined as an estimated glomerular filtration rate [eGFR] < 60 mL/min/1.73 m²) who received an infusion of 2 L isotonic saline in 4 hours. Ethylenediaminetetraacetic acid-plasma samples to measure FGF-23 concentrations were obtained immediately before and after administration of the infusion. The acute volume expansion study was approved by the local Medical Ethical Committee in Saarland, Germany. Furthermore, to investigate the long-term effects of modifications in volume status on FGF-23, we performed a post hoc analysis on a randomized, placebo-controlled, double-blinded cross-over intervention trial addressing the effects of sodium restriction and thiazide diuretic treatment during background ACEi. The original study protocol has been described previously (Dutch trial registry number 2366).[15] Briefly, 45 patients with type 2 diabetes and diabetic nephropathy (DN) were included. DN was defined as albuminuria > 30 mg/d, urinary albumin excretion > 20 mg/L, or urinary albumin-creatinine ratio > 2.5 mg/mmol for men and > 3.5 mg/mmol for women. Patients had a creatinine clearance > 30 mL/min and did not have signs of another primary renal disease. Main exclusion criteria were presence of type 1 diabetes, renovascular disease, a cardio- or cerebrovascular event < 3 months ago, overt hyperkalemia (> 6.0 mmol/L) or nephrotic syndrome, renal transplant recipients, use of immunosuppressants, blood pressure > 180/100 mm Hg, and contraindications to the use of lisinopril or HCT. All patients were titrated to maximum-dose ACEi (lisinopril 40 mg/d), with discontinuation of other rennin–angiotensin–aldosterone system-blockers/diuretics and stable dose of other antihypertensives. Patients underwent 4 subsequent 6-week treatments periods in random order: HCT (50 mg/d) or placebo, combined with either a sodium-restricted diet (targeting 50 mmol a day or ~3 g NaCl/d) versus a liberal sodium diet. Patients had 1 or 2 dietary counseling sessions with a dietitian and received a list with the sodium content of general used food products in the Netherlands. After each treatment period, measurements were performed, blood samples were taken by venipuncture, and 24-hour urine was collected. The study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethical Committee of the University Medical Center Groningen (protocol number 2010/288).

2.2. Laboratory measurements

For these analyses, we determined FGF-23 by human FGF-23 enzyme-linked immunosorbent assay directed against the carboxy-terminus (Immutopics Inc, San Clemente, CA; low cutoff 1.5 relative unit [RU]/mL; high cutoff 1500 RU/mL, intra-assay and interassay coefficients of variation of < 5% and < 16%, respectively[16]) in EDTA plasma samples obtained at baseline and after each treatment. Blood samples were stored at −80 °C and analyzed in batch. For 37 patients, EDTA-plasma was available for FGF-23 measurements at all 4 treatment periods. Blood and urinary electrolytes were measured by Roche Modular multianalyzer (Roche Diagnostics, Mannheim, Germany). Albuminuria was measured in 24-hour urine samples in single-batch by benzethonium chloride-based turbidimetric assay. Renin and aldosterone were measured with a chemiluminescence immunoassay (LIASON Aldosterone and LIASON Direct Renin, DiaSorin Deutschland GmbH, Dietzenbach, Germany). We calculated eGFR with the creatinine-based Chronic Kidney Disease Epidemiology Collaboration group equation.[17]
Baseline characteristics of the study population are depicted in Table 1. Median FGF-23 concentrations at baseline were 68 (58–76) pg/mL, the residuals of proteinuria were normally distributed after ln-transformation. In univariable regression analysis, FGF-23 was independently associated with ln-transformed residual proteinuria as dependent variable in univariate regression analysis as described earlier.\textsuperscript{[13]} Subsequently, we studied the relationship between FGF-23 and antiproteinuric response, and repeated these analyses with eGFR. We constructed multiplicative interaction terms for FGF-23 and proteinuria, creatinine clearance, and eGFR, respectively.

### 3. Results

#### 3.1. Volume loading and FGF-23 concentrations in hypertension

We first studied the effect of intravenous sodium loading on plasma FGF-23 in 12 hypertensive individuals without CKD stage 3 or higher, that is, with eGFR > 60 mL/min/1.73 m². These patients were 45 ± 13 years old and had normal renal function; further characteristics are presented in Table 1. Median FGF-23 plasma concentrations at baseline were 68 (58–97) RU/mL. The infusion of 2 L of saline in 4 hours did not change FGF-23 concentrations (P = 0.37, Fig. 1). Plasma renin concentration did not significantly change (from 4.5 [1.3–14.4] to 1.8 [0.8–9.6] RU/mL, P = 0.24), whereas aldosterone decreased significantly from 86 (70–140) to 58 (60–64) pg/mL as expected (P = 0.003).

#### 3.2. Volume reduction and FGF-23 concentrations in diabetic nephropathy

Baseline characteristics of the study population are depicted in Table 1. The DN patients were 65 ± 9 years old with a mean eGFR of 6.5 ± 2.5 mL/min/1.73 m² and proteinuria of 1.1 g/d (0.5–3.2 g/d). During ACEi monotherapy and RS diet, plasma FGF-23 concentration was 94 (73–141) RU/mL. Six weeks of treatment with add-on HCT did not significantly change the FGF-23 plasma concentration (posttreatment FGF-23 levels are presented in Table 2). Similarly, 6 weeks of add-on low-sodium (LS) diet did not affect FGF-23 plasma concentration. Combination therapy of both LS diet and HCT, in addition to ACEi treatment, resulted in a nonsignificant increase in FGF-23 to 111 (81–160) RU/mL (P = 0.15). Treatment with only HCT or a LS diet lowered proteinuria, as reported before,\textsuperscript{[11]} with a stronger proteinuric reduction after combination therapy, respectively (P < 0.001).

#### 3.3. FGF-23 and antiproteinuric response to volume interventions in DN

The residuals of proteinuria were normally distributed after ln-transformation. In univariable regression analysis, FGF-23 was independently associated with ln-transformed residual proteinuria in DN cohort. We first assessed the relationship between FGF-23 as independent and residual proteinuria as dependent variable in univariate regression analysis as described earlier.\textsuperscript{[13]} Subsequently, we studied the relationship between FGF-23 and residual proteinuria at the end of each treatment period in a model adjusted for “baseline” proteinuria, that is, proteinuria during regular sodium (RS) diet and placebo. Finally, we further adjusted for creatinine clearance, a potential confounder of the relation between FGF-23 and antiproteinuric response, and repeated these analyses with eGFR.

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### Table 1

| Baseline characteristics | HT patients, N=12 | DN patients, N=45 |
|-------------------------|------------------|------------------|
| Age, y                  | 45 ± 13          | 65 ± 9           |
| Male gender, n (%)      | 4 (33)           | 38 (84)          |
| BMI, kg/m²              | 28 ± 4           | 32 ± 5           |
| eGFR, mL/min/1.73 m²    | 103 ± 18         | 65 ± 25          |
| Creatinine, mg/d        | 17.8 ± 33        | 147 ± 16         |
| Proteinuria, g/d        | 0.1 (0.1–0.2)    | 1.1 (0.5–3.2)    |
| Albuminuria, mg/d       | 11 (7–14)        | 649 (230–2008)   |
| Systolic blood pressure, mm Hg | 178 ± 33 | 147 ± 16 |
| Diastolic blood pressure, mm Hg | 104 ± 18 | 82 ± 10 |
| Creatinine clearance, mL/min | 113 ± 27 | 101 ± 47 |
| Sodium excretion, mmol/24 h | 183 ± 61 | 224 ± 76 |
| HbA1c, %                | N/A              | 7.1 ± 0.8        |
| Diabetes duration, y    | N/A              | 11.8 ± 7.6       |
| Macrovascular disease, n (%) | 1 (8) | 21 (47) |
| Coronary artery disease, n (%) | 1 | 14 (31) |
| Stroke (CVA, TIA)       | 0                | 6 (13)           |
| Peripheral artery disease, n (%) | 0 | 7 (16) |
| Antihypertensives       |                  |                  |
| ACEi, n (%)             | 1 (8)            | 45 (100)         |
| Beta blocker, n (%)     | 0                | 27 (60)          |
| Alpha blocker, n (%)    | 4 (33)           | 4 (8)            |
| Calcium-channel blocker, n (%) | 5 (42) | 27 (60) |
| Diuretic therapy        | 0                | 0                |
| Vitamin D treatment, n (%) | 0 | 3 (7) |
| Phosphate binder treatment, n | 0 | 0 |

ACEi = angiotensin-converting enzyme inhibitor, BMI = body mass index, CVA = cerebrovascular accident, DN = diabetic nephropathy, eGFR = estimated glomerular filtration rate, HbA1c = glycated hemoglobin, HT = arterial hypertensive, TIA = transient ischemic attack.

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**Figure 1.** Effect of 2 L of saline infusion on fibroblast growth factor 23 concentrations after 4 hours. P value reflects Wilcoxon Signed Rank test. RU = relative unit.
significantly associated with residual proteinuria during RS diet and add-on HCT. The association was not significant during LS diet, but was again significant for the combination therapy of ACEi + HCT + LS (Table 3, model 1). In multivariable regression analysis, as expected, baseline proteinuria during RS diet outperformed FGF-23 as a correlate of proteinuria after add-on HCT (Table 3, model 2). Also during LS diet, FGF-23 was not significantly associated with the antiproteinuric response when adjusted for baseline proteinuria (Table 3). FGF-23 plasma concentrations were also not significantly correlated with the antiproteinuric response in the combination therapy group. When we used eGFR instead of creatinine clearance, this did not materially change the results. Invocation of multiplicative interaction terms did not demonstrate interaction between FGF23 and proteinuria, creatinine clearance, or eGFR, respectively (all P interaction >0.1).

4. Discussion

In the present study, we tested the hypothesis that volume intervention would impact FGF-23 concentrations in 2 independent settings, namely in patients with hypertension with preserved renal function and in DN patients. Such finding would support the existence of a negative feedback loop, where volume expansion could suppress FGF-23, while volume depletion could increase FGF-23 as counterpart regulatory response to FGF-23.

| Table 2 |
| Clinical parameters during different treatment periods. |

| Variables | Unit | ACEI + RS | ACEI + HCT + RS | ACEI + LS | ACEI + HCT + LS | Reference value | P |
|-----------|------|----------|----------------|----------|---------------|----------------|---|
| Clinical and general laboratory parameters | | | | | | | |
| Proteinuria | g/d | 1.1 (0.5–3.2) | 0.7 (0.5–1.7) | 0.7 (0.4–2.2) | 0.5 (0.3–1.3) | <0.3 | <0.001 |
| Proteinuria reduction | % | – | 27 (38) | 29 (26) | 46 (29) | <0.001 |
| Proteinuria/creatinine | g/mmol | 0.07 (0.04–0.22) | 0.04 (0.03–0.14) | 0.05 (0.03–0.18) | 0.03 (0.02–0.10) | <0.001 |
| Albuminuria | mg/d | 649 (230–2008) | 342 (215–1205) | 307 (147–1072) | 261 (125–878) | <0.001 |
| Systolic blood pressure | mm Hg | 147 ± 16 | 135 ± 16 | 141 ± 16 | 129 ± 14 | <0.140 |
| SBP reduction | % | – | 7.6 ± 8.0 | 3.4 ± 8.6 | 11.2 ± 8.3 | <0.001 |
| eGFR | mL/min/1.73 m² | 65 ± 25 | 69 ± 27 | 65 ± 27 | 59 ± 25 | <0.001 |
| Creatinine clearance | mL/min | 101 ± 47 | 97 ± 47 | 99 ± 48 | 88 ± 42 | 0.004 |
| Body weight | kg | 102 ± 18 | 100 ± 18 | 99 ± 18 | 98 ± 18 | <0.001 |
| 24-h sodium excretion | mmol/d | 224 ± 76 | 224 ± 88 | 148 ± 65 | 164 ± 71 | 0.100–250 |
| Plasma sodium | mmol/L | 140.3 ± 3.1 | 139.6 ± 3.2 | 139.7 ± 3.3 | 138.0 ± 3.7 | 135–145 |
| Mineral metabolism parameters | | | | | | | |
| FGF-23 | RIU/L | 94 (73–141) | 99 (74–148) | 99 (75–136) | 111 (81–160) | <0.125 |
| Serum phosphate | mmol/L | 1.00 ± 0.15 | 1.01 ± 0.14 | 1.01 ± 0.14 | 1.04 ± 0.18 | 0.70–1.50 |
| 24-h phosphate excretion | mmol/d | 25.8 ± 11.4 | 25.6 ± 10.4 | 22.9 ± 9.0 | 23.2 ± 9.7 | <0.480 |
| Serum calcium | mmol/L | 2.33 ± 0.11 | 2.34 ± 0.10 | 2.32 ± 0.10 | 2.36 ± 0.11 | 2.20–2.60 |
| 24-h calcium excretion | mmol/d | 1.6 (0.9–3.3) | 1.0 (0.4–2.6) | 1.3 (0.6–2.7) | 0.7 (0.4–1.7) | 2.5–7.5 |

FGF-23 and proteinuria were ln-transformed. All determinant variables are under ACEi + regular sodium diet conditions. 95% CI = 95% confidence interval, ACEi = angiotensin-converting enzyme inhibitor, B = unstandardized coefficient, CrCl = creatinine clearance, FGF-23 = fibroblast growth factor 23, HCT = hydrochlorothiazide, ln = natural log-transformed, LS = low-sodium diet. Standardized β = standardized coefficient.

4. Discussion

In the present study, we tested the hypothesis that volume intervention would impact FGF-23 concentrations in 2 independent settings, namely in patients with hypertension with preserved renal function and in DN patients. Such finding would support the existence of a negative feedback loop, where volume expansion could suppress FGF-23, while volume depletion could increase FGF-23 as counterpart regulatory response to FGF-23.

| Table 3 |
| Multivariable regression analysis for ln residual proteinuria after different treatments. |

| Treatment period | Model | Determinant | B | 95% CI B | Standardized β | P | R² |
|------------------|-------|-------------|---|-----------|----------------|---|-----|
| ACEI + HCT | 1 | FGF-23 | 0.582 | 0.133–1.030 | 0.412 | 0.01 | 0.17 |
| | 2 | FGF-23 | 0.277 | 0.016–0.538 | 0.196 | 0.04 | 0.75 |
| | 3 | FGF-23 | 0.233 | –0.046 to 0.513 | 0.165 | 0.10 | 0.75 |
| | | Proteinuria | 0.649 | 0.491–0.808 | 0.775 | <0.001 |
| | | CCl | –0.002 | –0.005 to 0.002 | –0.088 | 0.37 |
| ACEI + LS | 1 | FGF-23 | 0.456 | –0.124 to 1.035 | 0.264 | 0.12 | 0.07 |
| | 2 | FGF-23 | 0.029 | –0.233 to 0.291 | 0.017 | 0.82 | 0.83 |
| | 3 | FGF-23 | 0.086 | –0.192 to 0.364 | 0.050 | 0.53 | 0.84 |
| | | Proteinuria | 0.328 | 0.788–1.103 | 0.923 | <0.001 |
| | | CCl | 0.002 | –0.002 to 0.006 | 0.094 | 0.24 |
| ACEI + HCT + LS | 1 | FGF-23 | 0.528 | 0.024–1.031 | 0.343 | 0.04 | 0.12 |
| | 2 | FGF-23 | 0.220 | –0.132 to 0.572 | 0.143 | 0.21 | 0.61 |
| | 3 | FGF-23 | 0.270 | –0.199 to 0.648 | 0.175 | 0.16 | 0.62 |
| | | Proteinuria | 0.685 | 0.470–0.899 | 0.749 | <0.001 |
| | | CCl | 0.002 | –0.003 to 0.007 | 0.093 | 0.45 |
induced sodium retention. However, neither acute volume expansion nor chronic volume depletion changed FGF-23 concentrations.

Cardiovascular disease is highly prevalent in patients with CKD and the main cause of mortality in patients with CKD. Increased FGF-23 plasma concentrations are known to be independent predictors of adverse cardiovascular outcome in patients with CKD and in individuals with normal renal function. In these observational studies, FGF-23 was more compellingly associated with acute heart failure than with atherosclerotic events. Given the consistent associations between FGF-23 and markers of volume status in previous studies, and the implicated role for FGF-23 in volume homeostasis, we sought to investigate whether, conversely, an acute increase in volume status influences FGF-23 concentrations. We found that acute expansion of extracellular volume by sodium-chloride infusion did not reduce FGF-23 concentrations in patients with arterial hypertension.

This negative result may be explained by the short interval between the volume intervention and the FGF-23 measurement. In comparison, the increase of FGF-23 following dietary phosphate intake takes multiple hours to develop. On the other hand, acute changes in volume status such as cardiogenic shock are known to suddenly increase FGF-23 to far higher concentrations within a day and even on admission, respectively. Second, we also assessed the effects of chronic interventions, after homeostatic readjustment could have taken place. The DN patients had FGF-23 concentrations that are typically observed in patients with mildly impaired renal function. The volume-depleting interventions of dietary sodium restriction and HCT did not significantly increase FGF-23 concentrations in these patients. Intensification of treatment with HCT or a LS diet did not change FGF-23 concentrations. This is in line with our earlier observations in nondiabetic proteinuric CKD patients, where LS diet or add-on angiotensin-receptor blockade did not increase FGF-23 concentrations. However, combination of LS diet with HCT did show a small but nonsignificant increase in FGF-23 concentrations. This small increase is probably caused by the concomitant drop in renal function, as FGF-23 starts to increase markedly when renal function drops below ~60 mL/min/1.73 m². In that perspective, a greater increase in FGF-23 concentrations may have been expected. Of note, the observed decrease in renal function is considered an indicator of therapeutic efficacy, since such effect has been associated with long-term preserved renal function.

Our results suggest that the relationship between FGF-23 and volume status is not bidirectional in patients with mild-to-moderate CKD and in patients with normal renal function. The effects of FGF-23 on volume status as proposed by others may be considered an “off-target effect” of FGF-23, rather than that volume status triggers a FGF-23 response in CKD patients with mildly to moderately impaired renal function. In the setting of hemodialysis, on the other hand, the more extreme changes in volume status may result in a stronger correlation with FGF-23 concentrations.

Since both dietary sodium restriction and diuretic therapy reduced body weight and residual proteinuria, and volume depletion is known to potentiate the antiproteinuric response, we subsequently analyzed the relationship between FGF-23 and residual proteinuria. However, we could not demonstrate a strong relation of FGF-23 with the antiproteinuric response. This finding seems to vary with our earlier report, where a high FGF-23 concentration was correlated with an impaired antiproteinuric response to LS diet. In the present study, the correlation of FGF-23 with residual proteinuria was lost when we adjusted for renal function. An explanation may be that in the present study there were more patients with lower proteinuria levels (13 of 45 had proteinuria <0.5 g/dl, whereas in our previous study only 5 of 47 had proteinuria <0.5 g/dl). Patients with higher proteinuria reabsorb sodium more avidly, which makes sodium restriction a particular helpful strategy in these patients (as reviewed elsewhere). In addition, in the present study, the lower proteinuria levels before therapy intensification may have precluded the identification of determinants of proteinuria after therapy intensification in our regression analyses. Also, the smaller sample size likely led to less statistical power to detect an effect. Although nonsignificant, our present findings in patients with DN point toward a similar direction of effects as in our earlier report.

Strengths of our study include the use of a randomized controlled cross-over trial where we could assess 3 treatment combinations in the same subjects targeting volume status. Further, we performed a prospective experiment in patients who received intravenous sodium-chloride. FGF-23 plasma concentrations were determined using the same assay, enabling a comparison of the effects. Limitations of the study that deserve to be mentioned are the post hoc nature of the analysis in DN study and the small number of patients increasing the chance of a type II error (false negative finding), particularly in the infusion experiment. Therefore, larger experiments are needed to confirm our findings. Since phosphate intake was not controlled during the studies, changes in dietary phosphate might have influenced the results, although we did not observe any differences in urinary phosphate excretion or serum phosphate in response to volume interventions. In addition, in the infusion experiment, we investigated patients with arterial hypertension, who could have suffered from subclinical volume retention, which could have prevented a significant response of FGF-23 levels to acute expansion of extracellular volume.

Further, the interaction between FGF-23 and volume status appears to be stronger in proteinuric CKD, and proteinuria is a strong correlate of FGF-23 concentration, suggesting that the effects of fluid administration should be assessed in arterial hypertensive patients with proteinuria. The DN patients were all on background ACEi therapy. This may have altered the FGF-23–klotho–vitamin D axis, uncoupling the effect of increased renin expression by angiotensin 2 on FGF-23 concentrations, so that an additional effect of sodium restriction or HCT on FGF-23 plasma concentrations might have been precluded.

In conclusion, we could not demonstrate any effect of acute or chronic volume interventions on plasma FGF-23 concentrations in patients with DN or hypertension and normal renal function, respectively. Our data do not support a direct feedback mechanism of volume status on FGF-23. Future studies may address whether lowering of FGF-23 by other means in patients prone to volume retention improves outcomes.

References
[1] Isakova T, Wahl P, Vargas GS, et al. Fibroblast growth factor 23 is elevated before parathyroid hormone and phosphate in chronic kidney disease. Kidney Int 2011;79:1370–8.
[2] Scialla JJ, Wolf M. Roles of phosphate and fibroblast growth factor 23 in cardiovascular disease. Nat Rev 2014;10:268–78.
[3] Is JS, Katz R, Kestenbaum BR, et al. Fibroblast growth factor-23 and death, heart failure, and cardiovascular events in community-living...
individuals: CHS (Cardiovascular Health Study). J Am Coll Cardiol 2012;60:200–7.

[4] Lutsey PL, Alonso A, Selvin E, et al. Fibroblast growth factor-23 and incident coronary heart disease, heart failure, and cardiovascular mortality: the Atherosclerosis Risk in Communities study. J Am Heart Assoc 2014;3:e000936.

[5] Kestenbaum B, Sachs MC, Hoofnagle AN, et al. Fibroblast growth factor-23 and cardiovascular disease in the general population: the Multi-Ethnic Study of Atherosclerosis. Circ Heart Fail 2014;7:409–17.

[6] Faul C, Amaral AP, Oskouei B, et al. FGF23 induces left ventricular hypertrophy. J Clin Invest 2011;121:4393–408.

[7] Grabner A, Amaral AP, Schramm K, et al. Activation of cardiac fibroblast growth factor receptor 4 causes left ventricular hypertrophy. Cell Metab 2015;22:1020–32.

[8] Agarwal I, Ide N, Ix JH, et al. Fibroblast growth factor-23 and cardiac structure and function. J Am Heart Assoc 2014;3:e000384.

[9] Xie J, Yoon J, An S-W, et al. Soluble klotho protects against uremic cardiomyopathy independently of fibroblast growth factor 23 and phosphate. J Am Soc Nephrol 2015;65:259–60.

[10] Shalhoub V, Shatzen EM, Ward SC, et al. FGF23 neutralization reduces by haemodialysis. Nephrol Dial Transplant 2016;31:1494–501.

[11] Andrukhova O, Slavic S, Smorodchenko A, et al. FGF23 regulates renal sodium handling and blood pressure. EMBO Mol Med 2014;6:744–59.

[12] Baia LC, Humalda JK, Vervloet MG, et al. Fibroblast growth factor 23 and cardiovascular mortality after kidney transplantation. Clin J Am Soc Nephrol 2013;8:1968–78.

[13] Humalda JK, Baia LC, Kestenbaum B, et al. Fibroblast growth factor 23 is associated with proteinuria and smoking in chronic kidney disease. J Am Soc Nephrol 2014;25:349–60.

[14] Seiler S, Rogacev KS, Roth HJ, et al. Associations of FGF-23 and sKlotho with cardiovascular outcomes among patients with CKD stages 2–4. Clin J Am Soc Nephrol 2014;9:1049–58.

[15] Parker BD, Schurgers LJ, Brandenburg VM, et al. The associations of fibroblast growth factor 23 and uncarboxylated matrix Gla protein with mortality in coronary artery disease: the Heart and Soul Study. Ann Intern Med 2010;152:640–8.

[16] Udell JA, Morrow DA, Jarolim P, et al. Fibroblast growth factor-23, cardiovascular prognosis, and benefit of angiotensin-converting enzyme inhibition in stable ischemic heart disease. J Am Coll Cardiol 2014;63:2421–8.

[17] Voelzke B, Follath F, Parving HH, et al. Soluble ST2 and Galectin-3 for prognostication of cardiovascular death in heart failure patients. Int J Cardiol 2015;189:185–7.

[18] Gruson D, Lepoutre T, Ketelslegers J-M, et al. C-terminal FGF23 is a strong predictor of survival in systolic heart failure. Peptides 2012;37:258–62.

[19] Vogt L, Waanders F, Boomsma F, et al. Effects of dietary sodium and hydrochlorothiazide on the antiproteinuric efficacy of losartan. J Am Soc Nephrol 2008;19:999–1007.

[20] Svenningsen P, Friis UG, Versland JB, et al. Mechanisms of renal NaCl retention in proteinuric disease. Acta Physiol (Oxf) 2013;207:536–45.

[21] Heijboer AC, Levitus M, Vervloet MG, et al. Determination of fibroblast growth factor 23. Ann Clin Biochem 2009;46(pt 4):338–40.

[22] Levey AS, Stevens LA, Shmidt CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–12.

[23] Scailia JJ, Xie H, Rahman M, et al. Fibroblast growth factor-23 and cardiovascular events in CKD. J Am Soc Nephrol 2014;25:349–60.

[24] Vervloet MG, van Ittersum FJ, Riphagen IJ, et al. Fibroblast growth factor 23 and the antiproteinuric response to dietary sodium restriction and hydrochlorothiazide on RAAS blockade. Am J Kidney Dis 2012;60:200–7.

[25] Vervloet MG, van Ittersum FJ, Rutten RM, et al. Effects of dietary phosphate and calcium intake on fibroblast growth factor-23. Clin J Am Soc Nephrol 2011;6:383–9.

[26] Gruson D, Ferracin B, Ahn SA, et al. Comparison of fibroblast growth factor 23, soluble ST2 and Galectin-3 for prognostication of cardiovascular death in heart failure patients. Int J Cardiol 2015;189:185–7.

[27] Park J, Park E, Kim Y, et al. Association of FGF-23 and soluble ST2 with cardiovascular outcomes among patients with CKD stages 2–4. J Am Soc Nephrol 2014;9:1049–58.

[28] Saad MF, Devarajan P, Vanhalst S, et al. Fibroblast growth factor 23 and uncarboxylated matrix Gla protein with mortality in coronary artery disease: the Heart and Soul Study. Ann Intern Med 2010;152:640–8.

[29] Udell JA, Morrow DA, Jarolim P, et al. Fibroblast growth factor-23, cardiovascular prognosis, and benefit of angiotensin-converting enzyme inhibition in stable ischemic heart disease. J Am Coll Cardiol 2014;63:2421–8.

[30] Vervloet MG, van Ittersum FJ, Rutten RM, et al. Effects of dietary phosphate and calcium intake on fibroblast growth factor-23. Clin J Am Soc Nephrol 2011;6:383–9.

[31] Baia LC, Heilberg IP, Navis G, et al. Phosphate and FGF-23 homeostasis after kidney transplantation. Nat Rev 2015;11:656–66.

[32] Scialla JJ, Xie H, Rahman M, et al. Cross talk between the renin-angiotensin-aldosterone system and vitamin D-FGF-23-klotho in chronic kidney disease. J Am Soc Nephrol 2011;22:1603–9.