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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 2 L 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 (Immutopics) 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/1.73 m^2. 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 m^2 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 [81–160] 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, ln = natural logarithm, LS = low sodium, NCC = sodium-chloride cotransporter, RAAS = renin–angiotensin–aldosterone system, RS = regular sodium, RU = relative unit.

Keywords: CKD, fibroblast growth factor 23, hypertension, sodium, volume

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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. [2]). Similar associations between plasma FGF-23 concentrations and the incidence of decompensated heart failure were found among individuals without overt CKD.[3–5]

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] $<60\,mL/min/1.73\,m^2$) 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]

2.3. Statistical analyses

Statistical analyses were performed with SPSS Statistics 22 (IBM Corp., Armonk, NY). Data are reported as mean ± standard deviation or median (first to third quartile), as appropriate, after assessing normality by plotting the data. A P value <0.05 was considered to reflect statistical significance. Statistical significance of changes in FGF-23 plasma, aldosterone, and renin concentrations before and after sodium-chloride infusion were assessed by Wilcoxon Signed Rank test. In the DN cohort, data were natural log (ln)-transformed, as appropriate, and were compared by the Friedman test for dependent variables in case of skewed distribution. Normally distributed dependent variables were compared by one-way ANOVA with repeated measures. Multivariable regression analysis was performed to investigate the association of baseline FGF-23 with ln-transformed residual proteinuria after each of the 4 individual treatment periods in the 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.[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. 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 m^2. 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 isotonic 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] pg/mL, P = 0.24), whereas aldosterone decreased significantly from 86 (70–140) to 58 (0–64) 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 65 ± 25 mL/min/1.73 m^2 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,[11] with a stronger proteinuric reduction after combination therapy. Similarly, both individual and combined interventions influenced volume status, as reflected by a reduction in body weight (Table 2). Only combination therapy resulted in a decrease in creatinine clearance, whereas HCT treatment in itself also reduced eGFR.[11] Serum calcium and phosphate and urinary phosphate excretion did not differ across treatment periods (Table 2). Urinary calcium excretion dropped from 1.6 (0.9–3.3) mmol/d during RS diet and background ACEi therapy to 1.0 (0.4–2.6) mmol/d with add-on HCT, 1.3 (0.6–2.7) mmol/d with add-on LS diet and to 0.7 (0.4–1.7) mmol/d with combination therapy, respectively (P < 0.001).

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/m^2	28 ± 4	32 ± 5
eGFR, mL/min/1.73 m^2	101 ± 18	65 ± 25
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 (9)
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.

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

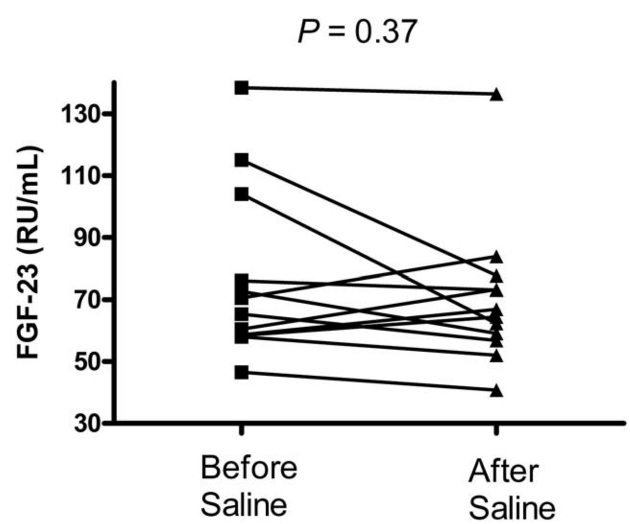

Figure 1. Effect of 2 L of saline infusion on fibroblast growth factor 23 concentrations after 4 hours. P value reflects Wilcoxon Signed Rank test. RU = relative unit.

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)	28 (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)	<30	<0.001
Systolic blood pressure	mm Hg	147 ± 16	135 ± 16	141 ± 16	129 ± 14	<140	<0.001
SBP reduction	%	–	7.6 ± 8.0	3.4 ± 8.6	11.2 ± 8.3		<0.001
eGFR	mL/min/1.73 m^2	65 ± 25	60 ± 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	100–250	<0.001
Plasma sodium	mmol/L	140.3 ± 3.1	139.6 ± 3.2	139.7 ± 3.3	138.0 ± 3.7	135–145	<0.001
Mineral metabolism parameters							
FGF-23	RU/mL	94 (73–141)	99 (74–148)	99 (75–135)	111 (81–160)	<125	0.15
Serum phosphate	mmol/L	1.00 ± 0.15	1.01 ± 0.14	1.01 ± 0.14	1.04 ± 0.18	0.70–1.50	0.31
24 h phosphate excretion	mmol/d	25.8 ± 11.4	25.6 ± 10.4	22.9 ± 9.0	23.2 ± 9.7	<48.0	0.13
Serum calcium	mmol/L	2.33 ± 0.11	2.34 ± 0.10	2.32 ± 0.10	2.36 ± 0.11	2.20–2.60	0.17
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	<0.001

ACEi = angiotensin-converting enzyme inhibitor, eGFR = estimated glomerular filtration rate, FGF-23 = fibroblast growth factor 23, HCT = hydrochlorothiazide, RS = regular sodium, SBP = systolic blood pressure.

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 HCT with LS diet (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 3

Multivariable regression analysis for ln residual proteinuria after different treatments.

Treatment period	Model	Determinant	B	95% CI B	Standardized β	*P*	R^2
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
		Proteinuria	0.663	0.508–0.818	0.790	<0.001	
	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	
		CrCl	–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
		Proteinuria	0.928	0.773–1.083	0.906	<0.001	
	3	FGF-23	0.086	−0.192 to 0.364	0.050	0.53	0.84
		Proteinuria	0.928	0.788–1.103	0.923	<0.001	
		CrCl	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
		Proteinuria	0.669	0.460–0.878	0.733	<0.001	
	3	FGF-23	0.270	−0.109 to 0.648	0.175	0.16	0.62
		Proteinuria	0.685	0.470–0.899	0.749	<0.001	
		CrCl	0.002	−0.003 to 0.007	0.093	0.45	

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.

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.[3,18–21] 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,[22–24] and the implicated role for FGF-23 in volume homeostasis,[11,13,22] 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.[25] 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.[26] 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.[13] 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 m^2.[1] 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.[27] 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.[14]

Since both dietary sodium restriction and diuretic therapy reduced body weight and residual proteinuria, and volume depletion is known to potentiate the antiproteinuric response,[28] 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 at variance with our earlier report, where a high FGF-23 concentration was correlated with an impaired antiproteinuric response to LS diet.[13] 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/d, whereas in our previous study only 5 of 47 had proteinuria <0.5 g/d). Patients with higher proteinuria reabsorb sodium more avidly,[29] which makes sodium restriction a particular helpful strategy in these patients (as reviewed elsewhere[30]). 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,[31] 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.[32]

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.

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