Original Article

Sodium restriction on top of renin–angiotensin–aldosterone system blockade increases circulating levels of *N*-acetyl-seryl-aspartyl-lysyl-proline in chronic kidney disease patients

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Objective: Sodium restriction potentiates the efficacy of the rennin–angiotensin–aldosterone system (RAAS)-blockade and improves long-term cardiovascular and renal protection, even independent of the better blood pressure control. The mechanisms underlying the potentiation of cardiorenal protection by sodium restriction are incompletely understood. RAAS-blockade with angiotensin-converting enzyme (ACE) inhibitors increases circulating levels of the anti-inflammatory and antifibrotic peptide *N*-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP), which is assumed to contribute to its therapeutic effects. We hypothesized that sodium restriction on top of RAAS-blockade further increases AcSDKP, as a possible explanation for the enhanced effects of RAAS-blockade during sodium restriction.

Methods: To test this hypothesis, we performed a secondary analysis of a randomized clinical trial investigating 46 nondiabetic chronic kidney disease (CKD) patients (age 50 ± 13 years, 80% men) with overt proteinuria and mild to moderate renal insufficiency. Patients were subjected, in a crossover design, to four double-blind 6-week study periods with either regular sodium diet (194 ± 49 mmol Na^{+}/day) or low sodium diet (102 ± 52 mmol Na^{+}/day) on top of either lisinopril (40 mg/day; single RAAS-blockade) or lisinopril plus valsartan (320 mg/day; dual RAAS-blockade).

Results: Sodium restriction significantly increased circulating levels of AcSDKP during single and dual RAAS-blockade ($P = 0.032$ and 0.042, respectively). Linear mixed-model analysis confirmed that AcSDKP levels were increased in response to sodium restriction, irrespective of sex, age, creatinine clearance, blood pressure, BMI, single or dual RAAS-blockade, treatment sequence and other dietary factors, that is calcium and protein ($P = 0.020$).

Conclusion: In patients with nondiabetic CKD, we demonstrated that sodium restriction, on top of single and dual RAAS-blockade, increases circulating levels of the anti-inflammatory and antifibrotic peptide AcSDKP. The rise in AcSDKP may contribute to the increased protection of RAAS-blockade during sodium restriction.

Keywords: chronic kidney disease, fibrosis, inflammation, *N*-acetyl-seryl-aspartyl-lysyl-proline, renoprotection, sodium restriction

Abbreviations: AcSDKP, N-Acetyl-Seryl-Aspartyl-Lysyl-Proline; RAAS, renin–angiotensin–aldosterone system; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin-II receptor antagonist

INTRODUCTION

Blockade of the rennin–angiotensin–aldosterone system (RAAS) by either angiotensin-converting enzyme inhibition (ACEi) or angiotensin-II receptor blockade (ARB) provides long-term cardiovascular and renal protection by reduction of blood pressure and proteinuria [1,2]. Sodium restriction is known to potentiate these beneficial effects of RAAS-blockade [3,4], resulting in substantially improved long-term cardiorenal protection [5,6]. Remarkably, the renoprotective effects of sodium restriction during RAAS-blockade can occur irrespective of blood pressure [3,5,7]. The mechanisms underlying this increased efficacy of RAAS-blockade during sodium restriction are incompletely understood, but may relate to a shift in the balance between vasoconstrictor and vasodilator angiotensins [8] and effects of sodium status on vascular and renal tissue ACE activity [9–11]. Furthermore, there are data suggesting that sodium restriction exerts renoprotection by anti-inflammatory and antifibrotic effects. This is

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supported by a reduction in urinary excretion of the fibrotic connective tissue growth factor (CTGF) in response to sodium restriction in addition to ARB in patients with nondiabetic chronic kidney disease (CKD) [12]. Moreover, experimental data suggest that glomerular influx of macrophages is decreased in response to sodium restriction and ACEi, independent of blood pressure [7]. In contrast, a high-sodium diet elicited a marked blood pressure-independent pro-fibrotic and pro-inflammatory response in both heart and kidneys [13].

N-Acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) is an endogenous tetrapeptide, present in the circulation as well as in kidney and heart tissue [14,15], with prominent anti-inflammatory and antifibrotic effects, as shown in experimental models of renal and cardiac disease [16–19]. Downregulation of AcSDKP elicits a pronounced stimulatory response on inflammation and fibrosis in the myocardium [20], whereas infusion of AcSDKP ameliorated the inflammatory and fibrotic damage induced by hypertension [21]. AcSDKP is specifically degraded by the N-terminal of ACE and its plasma levels rise substantially by inhibition of ACE, and accordingly, AcSDKP is thought to be involved in the therapeutic effects of RAAS-blockade, in particular in its anti-inflammatory and antifibrotic properties [22]. Whether sodium restriction during RAAS-blockade might result in an enhanced rise in AcSDKP, as a possible explanation for the enhanced efficacy during sodium restriction, is unknown. In the present study, we therefore investigated the effect of sodium restriction in CKD patients treated with single and dual RAAS-blockade on circulating levels of AcSDKP.

MATERIALS AND METHODS

Trial protocol

The current study is a secondary analysis among participants of a previously published clinical trial [4]. This clinical trial was a prospective randomized, double-blind, placebo-controlled cross-over trial in which the effects of ARB and sodium restriction on proteinuria and blood pressure were evaluated in nondiabetic proteinuric CKD patients. Inclusion criteria were age at least 18 years, proteinuria more than 1 g/day during high-dose ACEi, blood pressure more than 125/75 mmHg and creatinine clearance more than 30 ml/min. Exclusion criteria were SBP more than 180 mmHg, DBP more than 110 mmHg, diabetes mellitus (using WHO criteria), renovascular hypertension, decrease in creatinine clearance more than 6 ml/min in the previous year, a cardiovascular event in the previous 6 months, immunosuppressive treatment, regular use (>1 day/week) of NSAIDs, and pregnancy.

All CKD patients were enrolled in a run-in period of at least 6 weeks in which patients received standardized background treatment with ACEi at a maximum dose (lisinopril 40 mg/day), whereas all other RAAS-blocking agents were discontinued. Patients were subsequently treated with combinations of placebo, ARB (valsartan 320 mg/day), a regular sodium diet (target intake 200 mmol Na^+/day) and a low sodium diet (target intake 50 mmol Na^+/day), during four random 6-week study periods. Additional antihypertensive medication was allowed but kept stable throughout the trial. The drug interventions were double-blind, whereas the dietary interventions were open label.

Details on implementation of sodium restriction in this trial have been described previously [4]. In short, at inclusion, each patient received two to four dietary counselling sessions by professional dieticians. Individualized counselling used the general principle of remaining as close as possible to the patients' preferences and nutritional habits, to increase feasibility and compliance, taking into account adequacy of nutritional requirements as well as sodium content. Compliance to dietary sodium restriction was monitored by measuring urinary sodium excretion in 24-h urine samples in the middle and at end of each 6-week treatment period. Patients received extensive feedback on every 24-h urine collection.

In the original trial, 54 patients were enrolled of which 52 completed the trial and were included in primary analysis. The reasons for drop-out were either rash after initiation of ARB or lack of motivation to adhere to sodium restriction. In the current study, plasma samples for measurement of AcSDKP in all four treatment periods were available in 46 out of the 52 trial patients. Age, sex, proteinuria, creatinine clearance, blood pressure and BMI were not different between the 46 patients studied in the current study compared with the six patients not currently studied (data not shown).

Measurements and calculations

Patients visited the outpatient nephrology clinic at end of each 6-week treatment period for clinical assessment. Patients collected 24-h urine 1 day prior to their hospital visit in which we assessed proteinuria, nutritional intake (urinary excretion of sodium, potassium, calcium, phosphate and urea) and creatinine excretion, reflecting the accuracy of the 24-h urine collection. Blood pressure was measured for 15 min at 1-min intervals by a non-invasive automatic device (Dinamap; G.E. Medical Systems, Milwaukee, Wisconsin, USA), with patients being in a suspine position. We used the mean of the last thee readings. BMI was calculated by dividing body weight by height squared (kg/m^2). Mean arterial pressure (MAP) was calculated as diastolic pressure and one-third of pulse pressure. Renal function was estimated by calculating the endogenous clearance of creatinine.

Laboratory measurements

At end of each 6-week treatment period, blood was collected in EDTA-containing tubes (1.5 mg/ml) and placed immediately on ice upon blood withdrawal. Plasma was obtained by centrifugation at 3000*g* for 10 min at 4°C and stored at −80°C until analysis. AcSDKP was determined by competitive enzyme immunoassay (Caymann). Intraassay and interassay variation were below 6%. Proteinuria was measured in 24-h urine samples with a turbidimetric assay using benzethonium chloride (Modular; Roche Diagnostics, Mannheim, Germany). Serum and urine electrolytes and creatinine were measured using an automated multianalyser (Modular; Roche Diagnostics).

Statistical analysis

Data are shown as mean with standard deviation (SD) in case of normally distributed data and otherwise as median with interquartile range (IQR). Before statistical testing, skewed variables were log-transformed to obtain normality. Comparisons between different treatment periods were performed using paired *T*-tests. We used linear mixed-model analysis, with Bonferroni correction to adjust for multiple testing, to confirm univariate analysis by using the log-transformed values of AcSDKP as dependent variable, patients as a random factor, treatment allocation (using dummy variables with regular sodium and single RAAS-blockade as the reference group), treatment sequence and their interaction (treatment allocation∗sequence) as fixed factors, with sex, age, creatinine clearance, blood pressure, BMI and urinary urea and calcium excretion as covariates. Associations were tested using Pearson correlation test. As we found the association between AcSDKP and creatinine clearance to be exponential, we tested for presence of a significant association between these two by using linear regression analysis with the addition of quadratic transformed AcSDKP data. Data were analysed using SPSS version 18.0 (SPSS Inc., Chicago, Illinois, USA) and GraphPad Prism version 5 (GraphPad Software Inc., San Diego, California, USA). Two-sided *P*-value less than 0.05 was considered statistically significant.

RESULTS

We studied 46 nondiabetic CKD patients (age 50 ± 13 years, 80% men) with both primary and secondary renal disease (Table 1). Plasma renin and aldosterone levels at baseline were increased and decreased, respectively, suggesting good adherence to standardized background ACEi treatment. At baseline, by default, CKD patients were overtly proteinuric with mild to moderate renal insufficiency (Table 2). Urinary sodium excretion as a measure of dietary sodium intake decreased from 187 ± 53 and 182 ± 67 mmol/day during regular sodium diet to 106 ± 51 and 106 ± 60 mmol/day upon sodium restriction (during single and dual RAAS-blockade, respectively; both $P < 0.001$; Tables 2 and 3). In accordance, proteinuria, blood pressure and body weight were significantly decreased (all $P < 0.001$). Creatinine clearance was also significantly decreased upon sodium restriction ($P = 0.037$ and 0.007 for single and dual RAAS-blockade, respectively). During the periods with sodium restriction, urinary excretion of urea and calcium decreased as well. Urinary excretion of potassium, phosphate and creatinine remained stable throughout the trial.

TABLE 1. Patient characteristics ($n = 46$)

General parameters	
Male, *n* (%)	37 (80)
Caucasian race, *n* (%)	46 (100)
Age (years)	50 ± 13
BMI (kg/m^2)	27.4 ± 4.3
Creatinine clearance (ml/min)	83 ± 44
Nontrial antihypertensive medication	
α-blockade, *n* (%)	4 (9)
β-blockade, *n* (%)	8 (17)
Calcium channel blockade, *n* (%)	10 (22)
Diuretics, *n* (%)	12 (26)
Renal diagnosis	
Focal segmental glomerulosclerosis, *n* (%)	12 (26)
Immunoglobulin A nephropathy, *n* (%)	15 (33)
Membranous nephropathy, *n* (%)	6 (13)
Hypertensive nephropathy, *n* (%)	5 (11)
Other/inconclusive, *n* (%)	8 (17)
Plasma renin (ng/l)	61 (21–182)
Plasma aldosterone (nmol/l)	0.18 (0.12–0.37)

Local reference values for renin and aldosterone levels are 3.5–28.5 ng/l and 0.056–0.67 nmol/l, respectively.

TABLE 2. Clinical parameters and blood and urine measurements during regular and low sodium diet on top of single rennin–angiotensin–aldosterone system blockade (angiotensin-converting enzyme inhibitor) in patients with chronic kidney disease

Variables	RS + ACEi	LS + ACEi
Clinical parameters		
Creatinine clearance (ml/min)	83 ± 44	$76 \pm 45^{*}$
SBP (mmHg)	133 ± 20	$122 \pm 17^{**}$
DBP (mmHg)	80 ± 13	$73 \pm 11^{**}$
MAP (mmHg)	97 ± 15	$89 \pm 12^{**}$
Body weight (kg)	88.3 ± 17.6	$85.4 \pm 16.3^{**}$
Blood measurements		
Haemoglobin (mmol/l)	8.4 ± 0.8	8.4 ± 1.0
Leucocytes ($\times 10^9$/l)	7.1 ± 2.1	6.9 ± 2.2
Sodium (mmol/l)	141 ± 3	$139 \pm 3^{*}$
Potassium (mmol/l)	4.6 ± 0.6	$4.7 \pm 0.5^{*}$
Urine measurements		
Sodium excretion (mmol/day)	187 ± 53	$106 \pm 51^{**}$
Potassium excretion (mmol/day)	79 ± 25	75 ± 23
Calcium excretion (mmol/day)	1.1 (0.1–2.3)	0.8 (0.4–1.3)**
Phosphate excretion (mmol/day)	32 ± 11	29 ± 10
Urea excretion (mmol/day)	389 ± 111	$358 \pm 115^{*}$
Creatinine excretion (mmol/day)	13.8 ± 4.2	13.4 ± 4.2
Protein excretion (g/day)	1.9 (0.9–3.0)	0.8 (0.5–1.2)**

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin-II receptor antagonist; DBP, diastolic blood pressure; LS, low sodium diet; MAP, mean arterial pressure; RS, regular sodium diet; SDP, systolic blood pressure.
$^{*}P < 0.05$ vs. RS + ACEi.
$^{**}P \leq 0.001$ vs. RS + ACEi.

TABLE 3. Clinical parameters and blood and urine measurements during regular and low sodium diet on top of dual rennin–angiotensin–aldosterone system blockade (angiotensin-converting enzyme inhibitor and angiotensin-II receptor antagonist) in patients with chronic kidney disease

Variables	RS + ACEi + ARB	LS + ACEi + ARB
Clinical parameters		
Creatinine clearance (ml/min)	84 ± 43	70 ± 42*
SBP (mmHg)	131 ± 24	119 ± 18**
DBP (mmHg)	77 ± 14	70 ± 14**
Mean arterial pressure (mmHg)	95 ± 16	87 ± 14**
Body weight (kg)	87.4 ± 16.5	85.5 ± 16.8**
Blood measurements		
Haemoglobin (mmol/l)	8.5 ± 1.0	8.2 ± 1.0*
Leucocytes ($\times 10^9$/l)	7.1 ± 2.2	7.1 ± 2.3
Sodium (mmol/l)	141 ± 2	139 ± 3**
Potassium (mmol/l)	4.6 ± 0.7	5.0 ± 0.7**
Urine measurements		
Sodium excretion (mmol/day)	182 ± 67	106 ± 60**
Potassium excretion (mmol/day)	77 ± 25	75 ± 22
Calcium excretion (mmol/day)	0.7 (0.5–2.2)	0.6 (0.3-1.3)**
Phosphate excretion (mmol/day)	29 ± 10	28 ± 11
Urea excretion (mmol/day)	395 ± 106	352 ± 124*
Creatinine excretion (mmol/day)	14.0 ± 4.0	13.5 ± 4.7
Protein excretion (g/day)	1.3 (0.6–3.2)	0.5 (0.3–1.3)**

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin-II receptor antagonist; DBP, diastolic blood pressure; LS, low sodium diet; MAP, mean arterial pressure; RS, regular sodium diet; SDP, systolic blood pressure.
*$P < 0.05$ vs. RS + ACEi + ARB.
**$P \leq 0.001$ vs. RS + ACEi + ARB.

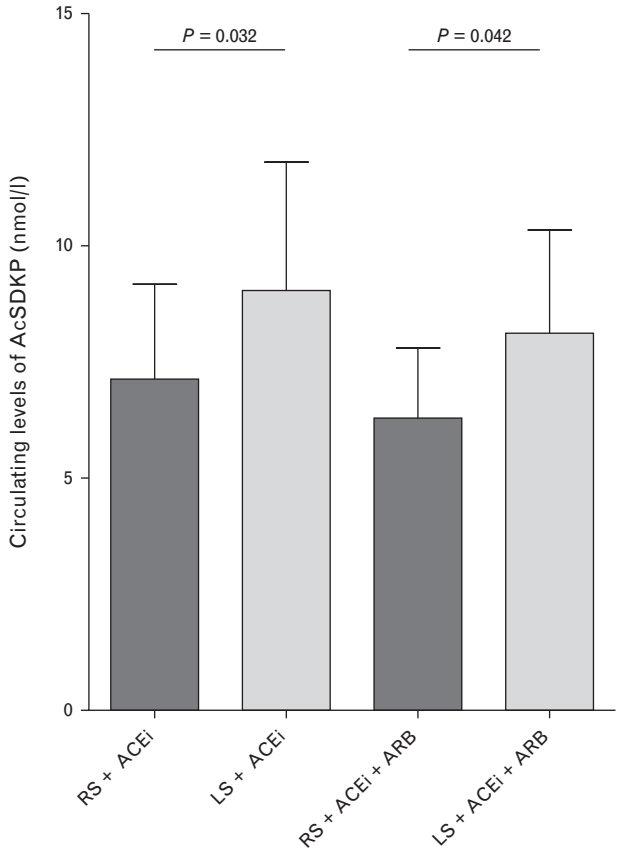

FIGURE 1 Circulating levels of *N*-acetyl-seryl-aspartyl-lysyl-proline during regular (RS) and low sodium diet (LS) on top of single (ACEi) and dual RAAS-blockade (ACEi and ABR) in patients with chronic kidney disease. ACEi, angiotensin-converting enzyme inhibitor; AcSDKP, *N*-acetyl-seryl-aspartyl-lysyl-proline; ARB, angiotensin-II receptor antagonist; LS, low sodium diet; RS, regular sodium diet.

Circulating levels of AcSDKP were significantly increased in response to 6-week sodium restriction ($P = 0.032$ and 0.042 for single and dual RAAS-blockade, respectively; Fig. 1). Furthermore, AcSDKP was significantly associated with urinary sodium excretion during sodium restriction diet ($R = -0.297$, $P = 0.045$ and $R = -0.346$, $P = 0.020$ during single and dual RAAS-blockade, respectively). AcSDKP was not affected by sex ($P = 0.5$) nor by dual RAAS-blockade as compared with monotherapy ACEi ($P = 0.41$ and $P = 0.29$ for regular and low sodium diet, respectively). Creatinine clearance was inversely and exponentially correlated with plasma AcSDKP levels during regular sodium diet ($\beta = -42$, $P = 0.023$ and $\beta = -99$, $P = 0.003$, for single and dual RAAS-blockade, respectively) and sodium restriction ($\beta = -59$, $P = 0.021$ and $\beta = -67$, $P = 0.002$ for single and dual RAAS-blockade, respectively). Furthermore, change in creatinine clearance induced by sodium restriction was associated with a change in plasma AcSDKP during both single and dual RAAS-blockade ($R = -0.307$, $P = 0.045$ and $R = -0.562$, $P < 0.001$, respectively; Fig. 2). To test whether the increase in AcSDKP during sodium restriction was independent of other factors, we performed multivariate analysis, demonstrating that indeed the rise in AcSDKP was independent of creatinine clearance, sex, age, blood pressure, BMI, single or dual RAAS-blockade, treatment sequence, and urinary urea and calcium excretion ($P = 0.028$). The estimated marginal mean for sodium restriction for log-transformed AcSDKP was 0.121 [95% confidence interval 0.014–0.229].

DISCUSSION

We demonstrated that sodium restriction, on top of single and dual RAAS-blockade in nondiabetic proteinuric CKD patients, independently increases circulating levels of the

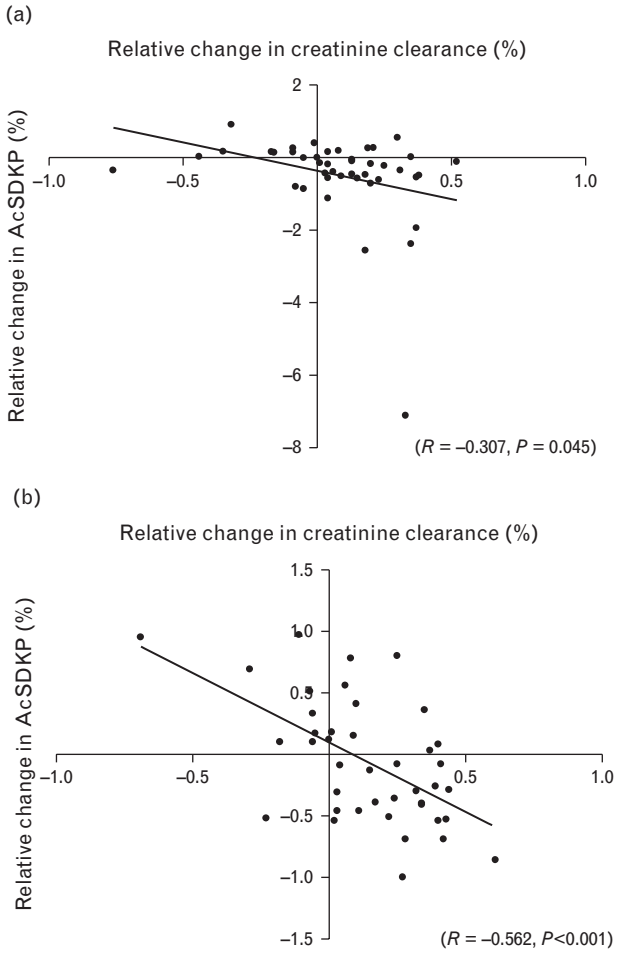

FIGURE 2 Scatter plots showing the univariate associations between change in creatinine clearance and change in circulating *N*-acetyl-seryl-aspartyl-lysyl-proline induced by low sodium diet on top of treatment with ACEi (a), and ACEi and ARB (b) in patients with chronic kidney disease. ACEi, angiotensin-converting enzyme inhibitor; AcSDKP, *N*-acetyl-seryl-aspartyl-lysyl-proline; ARB, angiotensin-II receptor antagonist.

anti-inflammatory and antifibrotic peptide AcSDKP. We propose that this increase in AcSDKP might contribute to the effects of sodium restriction on the therapeutic efficacy of RAAS-blockade in CKD patients.

Several publications report upon the anti-inflammatory and antifibrotic properties of AcSDKP. As to its anti-inflammatory aspects, AcSDKP was found to inhibit inflammation in experimental models of kidney, heart and liver disease [23–27]. Although the precise mechanism of inhibition of inflammation by AcSDKP is not well characterized, blockade of monocyte cytokine monocyte chemoattractant protein-1 (MCP-1), possibly through blockade of the transcription factor nuclear factor-kappa B (NF-κB), has been suggested [17]. Of note, as AcSDKP has negative regulatory effects on proliferation of haematopoietic stem cells [28], the antifibrotic action of AcSDKP may also be partially attributed to inhibition of leukocyte number or differentiation.

Although inflammation in itself is a well established factor in initiation and progression of fibrosis [29,30], AcSDKP was reported to have direct independent effects on tissue fibrosis as well. Treatment with AcSDKP inhibited renal damage in experimental models of diabetic kidney disease, 5/6 nephrectomy and unilateral ureteral obstruction [17,31,32]. Furthermore, in a model of anti-GBM nephritis, infusion of AcSDKP ameliorated progression of renal insufficiency by retarding glomerulosclerosis and tubulointerstitial fibrosis [23]. Importantly, these effects were all independent of blood pressure. On the contrary, decreased endogenous levels of AcSDKP promoted excessive collagen deposition in the kidney and heart [19,20]. The mechanism of action of AcSDKP is probably, in part, by inhibition of transforming growth factor beta (TGFβ) signalling by blocking the Smad-pathway [20].

Rise in AcSDKP as an anti-inflammatory and antifibrotic factor is well in line with data demonstrating anti-inflammatory and antifibrotic effects of sodium restriction [7,12]. The beneficial effect of sodium restriction upon inflammation and fibrosis in CKD is supported by the reduction in urinary excretion of fibrotic CTGF [12]. Furthermore, experimental data suggest that glomerular influx of macrophages is decreased in response to sodium restriction and ACEi [7]. In contrast, a high-sodium diet elicited a marked blood pressure independent pro-fibrotic response in both heart and kidneys [13]. High sodium intake was reported to have adverse effects on vascular function as well [33,34], and sodium restriction reversed the age and hypertension-associated endothelial dysfunction, irrespective of blood pressure response [35].

What mechanisms could account for the rise in AcSDKP upon sodium restriction on top of RAAS-blockade? As this report is the first clinical study investigating AcSDKP with regard to sodium restriction, one can only speculate on the exact mechanisms of AcSDKP regulation. First, AcSDKP is exclusively degraded by ACE. Several reports documented a reduced tissue ACE activity upon sodium restriction [36–39], so decreased renal tissue ACE activity in response to sodium restriction might be responsible for higher AcSDKP levels. Second, AcSDKP is locally released from its precursor thymosine-β4, most likely by prolyl oligopeptidase (POP), a serine proteinase found in mammalian tissues. POP in turn is negatively regulated by alpha-1-antitrypsine, which is abundantly present in mast cells, which infiltrate and granulate following acute and chronic injury [40]. As sodium restriction is, as stated previously, associated with reduction in inflammation and fibrosis, POP activity might be elevated resulting in increased levels of AcSDKP. Alternatively, oxidative stress, which is significantly increased in kidney fibrosis, can activate POP [41], and interestingly, sodium restriction was found to reduce this oxidative stress [42]. Finally, AcSDKP was related to creatinine clearance, probably because its route of elimination is primarily renal, and sodium restriction in our trial reduced renal function, and thereby, possibly AcSDKP elimination. However, the decrease in creatinine clearance could not fully explain the rise in AcSDKP as adjustment for creatinine clearance in linear mixed modelling did not eliminate the association between sodium restriction and AcSDKP.

The decrease in renal function in response to sodium restriction during RAAS-blockade is in line with previous studies [3,43], and most likely reflects a reversible reduction

in intraglomerular pressure [44]. In our trial, indeed, it was reversible upon discontinuation of the sodium restriction. In the long term, the short-term decrease in renal function at onset of therapy predicts a better renal outcome, attributed to lower glomerular pressure [45,46]. We now show that the short-term reduction in renal function during intensification of therapy, by sodium restriction, is associated with an increase in circulating AcSDKP during single and dual RAAS-blockade, which might provide an additional explanation for the favourable prognostic impact of a short-term fall in renal function during renoprotective therapy. In that respect, AcSDKP might be regarded as a 'positive' uremic toxin. This is not unique: for example, the anti-inflammatory cytokine adiponectin was found to be inversely associated with renal function [47].

Dual blockade may have detrimental effects (for instance as observed in the ONTARGET trial [48] and the recently published ALTITUDE trial [49]). In our trial, addition of ARB to ACEi was not associated with an increase in plasma AcSDKP levels. This was as expected considering that AcSDKP is exclusively metabolized by ACE. Dual RAAS-blockade was not associated with an adverse effect on renal function in this trial, although a possible detrimental effect on the long-term cannot be excluded. Furthermore, dual RAAS-blockade only had a small effect on blood pressure, which is in line with a previous meta-analysis [50], possible due to an inappropriate rise in circulating renin levels [51].

This study has several limitations. First, all liabilities associated with secondary analysis apply. Second, this study should be considered as hypothesis-generating, as the exact mechanism on how sodium restriction affects circulating AcSDKP levels is not known. It should be noted that the significant and inverse association between sodium excretion – as a measure of sodium intake – and AcSDKP during sodium restriction enhances the robustness of our finding that AcSDKP was increased in response to our intervention in sodium status. Third, we have measured AcSDKP neither in healthy individuals without renal impairment nor in those with renal impairment but without RAAS-blockade. We can therefore not state whether or to which extent levels of AcSDKP were increased in this population at baseline. However, much lower levels of AcSDKP were reported in healthy controls with normal renal function and without treatment with RAAS-blockade using the same protocol and ELISA kit [52]. Furthermore, ACEi is known to increase levels of AcSDKP four to five-fold compared with baseline [53,54]. Fourth, we did not measure AcSDKP during additional standardized time points throughout the trial other than at the end of each 6-week treatment period, for instance during the treatment periods or after completion of the trial. Consequently, we do not have data on the kinetics of the response rate of AcSDKP or the sustainability of its response to sodium restriction. However, at any rate, the response of AcSDKP was sustained at 6 weeks of follow-up. Fifth, it would be of interest to explore the downstream effects of ASDKP by studying its relation to circulating fibrotic and inflammation markers; however, these data were not available in our trial patients. Finally, this was a short-term study, which means that further research is needed to investigate the long-term effects of sodium restriction on AcSDKP.

In conclusion, in white patients with nondiabetic CKD, we found that circulating levels of AcSDKP significantly and independently increased in response to sodium restriction on top of single and dual RAAS-blockade. We postulate that upregulation of AcSDKP might contribute to the enhanced renoprotective efficacy of RAAS-blockade during moderate sodium restriction.

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Conflicts of interest

The authors have no potential conflicts of interest.

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Reviewers' Summary Evaluations

Referee 1

This is a nice work on a very interesting topic, and may stimulate further studies. First, to confirm the data in a different population (non-Caucasian) and on patients with diabetic chronic kidney disease, which is nowadays the most common cause of end stage CKD. Second, to investigate whether the response to RAS blockade may be different between patients with and without renal insufficiency. Finally, it would be useful to know other factors associated with proteinuria in this population, such as BMI, and LDL-cholesterol levels, which may have interfered with the response to RAS blockade.

Referee 2

N-Acetyl-Seryl-Aspartyl-Lysyl-Proline (AcSDKP)is an antifibrotic and anti-inflammatory peptide that has been shown to be increased in the setting of ACE inhibitor (ACEi) use. In this report, the authors measured AcSDKP levels in patients with proteinuric CKD with mild to moderate decrements in renal function, who took part in a cross-over RCT of the addition of ARB/placebo, low sodium/regular sodium intake, to maximal ACEi therapy. Their findings reveal that AcSDKP levels are significantly elevated in the setting of sodium restriction (in addition to max. ACEi or ACEi/ARB) and this is hypothesis generating (i.e. AcSDKP could mediate some of the benefits of sodium restriction in the setting of ACEi treatment).