Pathophysiological aspects of lifestyle and the kidney
Kwakernaak, Adriaan Johan

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2014

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
CHAPTER 11

Sodium restriction on top of RAAS blockade increases circulating levels of n-acetyl-seryl-aspartyl-lysyl-proline in chronic kidney disease patients

Arjan J. Kwakernaak, Femke Waanders, Maartje C.J. Slagman, Martin M. Dokter, Gozewijn D. Laverman, Rudolf A. de Boer, Gerjan Navis

J Hypertens 2013; 31: 2425-2432
ABSTRACT

Introduction. Sodium restriction potentiates the efficacy of renin-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 cardio-renal protection by sodium restriction are incompletely understood. RAAS blockade with 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.

Material and Methods. To test this hypothesis, we performed a secondary analysis of a randomized clinical trial investigating 46 chronic kidney disease (CKD) patients (age 50±13 years, 80% male) with overt proteinuria and mild-to-moderate renal insufficiency. Patients were subjected, in a crossover design, to 4 double-blind 6-week 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/d; single RAAS blockade) or lisinopril plus valsartan (320 mg/d; 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 gender, age, creatinine clearance, blood pressure, BMI, single or dual RAAS blockade, treatment sequence, and other dietary factors, i.e. calcium and protein (P=0.020).

Conclusion. In Caucasian patients with non-diabetic CKD, we demonstrate 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.
INTRODUCTION
Blockade of the renin-angiotensin-aldosterone system (RAAS) by either angiotensin converting enzyme inhibition (ACEi) or angiotensin receptor blockade (ARB) provides long-term cardiovascular and renal protection by reduction of blood pressure and proteinuria. Sodium restriction is known to potentiate these beneficial effects of RAAS blockade, resulting in substantially improved long-term cardio-renal protection. Remarkably, the renoprotective effects of sodium restriction during RAAS blockade can occur irrespective of blood pressure. 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 and effects of sodium status on vascular and renal tissue ACE activity. Furthermore, there are data suggesting that sodium restriction exerts renoprotection by anti-inflammatory and antifibrotic effects. This is supported by reduced urinary excretion of the fibrotic connective tissue growth factor in response to sodium restriction in addition to ARB in patients with non-diabetic chronic kidney disease (CKD). Moreover, experimental data suggests that glomerular influx of macrophages is decreased in response sodium restriction and ACEi, independent of blood pressure. In contrast, a high sodium diet elicited a marked blood pressure-independent pro-fibrotic and pro-inflammatory response in both heart and kidneys.

n-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) is an endogenous tetrapeptide, present in the circulation as well as in kidney and heart tissue, with prominent anti-inflammatory and antifibrotic effects, as shown in experimental models of renal and cardiac disease. Downregulation of AcSDKP elicits a pronounced stimulatory response on inflammation and fibrosis in the myocardium, whereas infusion of AcSDKP ameliorated the inflammatory fibrotic damage induced by hypertension. AcSDKP is specifically degraded by the N-terminal of ACE and its plasma levels rises substantially by inhibition of ACE, and accordingly, AcSDKP is thought to be involved in the therapeutic effects of RAAS blockade, in particular its anti-inflammatory and antifibrotic properties. 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.

MATERIAL AND METHODS
Trial protocol
The current study is a secondary analysis among participants of a previously published clinical trial. 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 non-diabetic proteinuric CKD patients. Inclusion
criteria were age ≥ 18 years, proteinuria > 1 g/d during high-dose ACEi, blood pressure > 125/75 mmHg and creatinine clearance ≥ 30 mL/min. Exclusion criteria were systolic blood pressure ≥ 180 mmHg, diastolic blood pressure ≥ 110 mmHg, diabetes mellitus (using World Health Organization criteria), renovascular hypertension, decrease in creatinine clearance ≥ 6 mL/min in the previous year, a cardiovascular event in the previous 6 months, immunosuppressive treatment, regular use (>1 d/week) of non-steroidal anti-inflammatory drugs, 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 maximum dose (lisinopril 40 mg/d) while all other RAAS blocking agents were discontinued. Patients were subsequently treated with combinations of placebo, ARB (valsartan 320 mg/d), a regular sodium diet (target intake 200 mmol Na+/d) and a low sodium diet (target intake 50 mmol Na+/d), during four random 6-week trial 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.

Specific details on implementation of sodium restriction in this trial have been described in detail previously. In short, at inclusion, each patient received two to four dietary counseling sessions by professional dietitians. Individualized counseling 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 hour urine samples in the middle and at end of each 6-week treatment period. Patients received extensive feedback on every 24 hour 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 4 treatment periods were available in 46 out of the 52 trial patients. Age, gender, proteinuria, creatinine clearance, blood pressure and BMI were not different between the 46 patients studied in the current study compared to the 6 patients not currently studied (data not shown).

**Measurements and calculations**

CKD patients visited the nephrology outpatient clinic at end of each 6-week treatment period for clinical assessment. Patients collected 24 hour urine one 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 hour urine collection. Blood pressure was measured for 15 minutes at 1-minute intervals by a non-invasive automatic device (Dinamap®, G.E. Medical Systems, Milwaukee, WI, USA),
with patients being in a supine position. We used the mean of the last three readings. Body mass index (BMI) was calculated by dividing body weight by height squared (kg/m²). Mean arterial pressure (MAP) was calculated as diastolic pressure plus one third of pulse pressure. Renal function was estimated by calculating the endogenous clearance of creatinine.

**Laboratory measurements**

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 40°C and stored at −80°C until analysis. AcSDKP was determined by competitive enzyme immunoassay (Caymann). Intra- and inter assay variation were below 6%. Proteinuria was measured in 24 hour urine samples with a turbidimetric assay using benzethonium chloride (Modular, Roche Diagnostics, Mannheim, Germany). Serum and urine electrolytes and creatinine was measured using an automated multianalyser (Modular, Roche Diagnostics, Mannheim, Germany).

**Statistical analysis**

Data are given 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 natural 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, participants 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 gender, age, creatinine clearance, blood pressure, BMI, and urinary urea and calcium excretion as covariates. Univariate 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 additional adjustment with quadratic and log-transformed AcSDKP data. Data was analyzed using SPSS version 18.0 (SPSS Inc., Chicago, IL) and GraphPad Prism version 5 (GraphPad Software Inc., San Diego, CA). Two-sided P-value <0.05 was considered statistically significant.

**RESULTS**

We studied 46 CKD patients (age 50±13 years, 80% male) with both primary and secondary renal disease (Table 1). At baseline, by default, CKD patients were overtly proteinuric with mild to moderate renal insufficiency (Table 2A). Plasma renin and aldosterone levels at baseline were increased and decreased, respectively, suggesting good adherence to
standardized background ACEi treatment. Urinary sodium excretion as a measure of
dietary sodium intake decreased from 187±53 and 182±67 mmol/d during regular sodium
diet to 106±51 and 106±60 mmol/d upon sodium restriction (during single and dual RAAS
blockade, respectively; both P<0.001; Table 2A and 2B). 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.

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; Figure 1).
Furthermore, AcSDKP was significantly associated with urinary sodium excretion during
low sodium diet (R=-0.297, P=0.045 and R=-0.346, P=0.020 during single and dual RAAS
blockade, respectively). AcSDKP was not effected by gender (P=0.5) nor by dual RAAS
blockade as compared to 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 (β=-42, P=0.023 and β=-99, P=0.003,
for single and dual RAAS blockade, respectively) and sodium restriction (β=-59, P=0.021
and β=-67, P=0.002 for single and dual RAAS blockade, respectively). Furthermore, change
in creatinine clearance induced by dietary sodium restriction was associated with change

Table 1. Patient characteristics (n=46).

<table>
<thead>
<tr>
<th>General parameters</th>
<th>Male, n (%)</th>
<th>37 (80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian race, n (%)</td>
<td>46 (100)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>50±13</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.4±4.3</td>
<td></td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>83±44</td>
<td></td>
</tr>
<tr>
<td>Non-trial antihypertensive medication:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-blockade, n (%)</td>
<td>4 (9)</td>
<td></td>
</tr>
<tr>
<td>β-blockade, n (%)</td>
<td>8 (17)</td>
<td></td>
</tr>
<tr>
<td>Calcium channel blockade, n (%)</td>
<td>10 (22)</td>
<td></td>
</tr>
<tr>
<td>Diuretics, n (%)</td>
<td>12 (26)</td>
<td></td>
</tr>
<tr>
<td>Renal diagnosis:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal segmental glomerulosclerosis, n (%)</td>
<td>12 (26)</td>
<td></td>
</tr>
<tr>
<td>Immunoglobulin A nephropathy, n (%)</td>
<td>15 (33)</td>
<td></td>
</tr>
<tr>
<td>Membranous nephropathy, n (%)</td>
<td>6 (13)</td>
<td></td>
</tr>
<tr>
<td>Hypertensive nephropathy, n (%)</td>
<td>5 (11)</td>
<td></td>
</tr>
<tr>
<td>Other/inconclusive, n (%)</td>
<td>8 (17)</td>
<td></td>
</tr>
<tr>
<td>Plasma renin (ng/L)</td>
<td>61 (21-182)</td>
<td></td>
</tr>
<tr>
<td>Plasma aldosterone (nmol/L)</td>
<td>0.18 (0.12-0.37)</td>
<td></td>
</tr>
</tbody>
</table>

Local reference values for renin and aldosterone levels are 3.5-28.5 ng/L and 0.056-0.67
nmol/L, respectively.
SODIUM RESTRICTION AND AcSDKP

**DISCUSSION**

We demonstrate that sodium restriction, on top of single and dual RAAS blockade in non-diabetic proteinuric CKD patients, independently increases circulating levels of the 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 plasma AcSDKP during both single and dual RAAS blockade (R=-0.307, P=0.045 and R=-0.562, P<0.001, respectively; Figure 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, gender, 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).

**Table 2A: Clinical parameters during regular (RS) and low sodium diet (LS) on top of single RAAS blockade (ACEi) in CKD patients.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>RS + ACEi</th>
<th>LS + ACEi</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical parameters:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>83±44</td>
<td>76±45*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>133±20</td>
<td>122±17**</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80±13</td>
<td>73±11**</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>97±15</td>
<td>89±12**</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>88.3±17.6</td>
<td>85.4±16.3**</td>
</tr>
<tr>
<td><strong>Blood measurements:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (mmol/L)</td>
<td>8.4±0.8</td>
<td>8.4±1.0</td>
</tr>
<tr>
<td>Leucocytes (x10^9/L)</td>
<td>7.1±2.1</td>
<td>6.9±2.2</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>141±3</td>
<td>139±3*</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.6±0.6</td>
<td>4.7±0.5*</td>
</tr>
<tr>
<td><strong>Urine measurements:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium excretion (mmol/d)</td>
<td>187±53</td>
<td>106±51**</td>
</tr>
<tr>
<td>Potassium excretion (mmol/d)</td>
<td>79±25</td>
<td>75±23</td>
</tr>
<tr>
<td>Calcium excretion (mmol/d)</td>
<td>1.1 (0.1-2.3)</td>
<td>0.8 (0.4-1.3)**</td>
</tr>
<tr>
<td>Phosphate excretion (mmol/d)</td>
<td>32±11</td>
<td>29±10</td>
</tr>
<tr>
<td>Urea excretion (mmol/d)</td>
<td>389±111</td>
<td>358±115*</td>
</tr>
<tr>
<td>Creatinine excretion (mmol/d)</td>
<td>13.8±4.2</td>
<td>13.4±4.2</td>
</tr>
<tr>
<td>Protein excretion (g/d)</td>
<td>1.9 (0.9-3.0)</td>
<td>0.8 (0.5-1.2)**</td>
</tr>
</tbody>
</table>

* P≤0.05 vs. RS + ACEi, ** P≤0.001 vs. RS + ACEi. Abbreviations: RS: regular sodium diet, LS: low sodium diet, ACEi: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker.
experimental models of kidney, heart and liver disease. Although the precise mechanism of inhibition of inflammation by AcSDKP is not well-characterized, blockade of monocyte cytokine monocyte chemoattractant proptein-1 (MCP-1), possibly through blockade of the transcription factor nuclear factor-kappa B (NF-B), has been suggested. Of note, as AcSDKP has negative regulatory effects on proliferation of hematopoietic stem cells, 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, 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. Furthermore, in a model of anti-GBM nephritis, infusion of AcSDKP ameliorated progression of renal insufficiency by retarding glomerulosclerosis and tubulo-interstitial fibrosis. Importantly, these effects were all independent of blood pressure. On the other hand, decreased endogenous levels of AcSDKP promoted excessive collagen deposition in the kidney and heart. The mechanism of action of AcSDKP is probably, in part, by inhibition of transforming growth factor beta (TGF) signaling by blocking the Smad-pathway.
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.\textsuperscript{7,12} The beneficial effect of sodium restriction upon inflammation and fibrosis in CKD is supported by the reduction in urinary excretion of fibrotic connective tissue growth factor (CTGF).\textsuperscript{12} Furthermore, experimental data suggests that glomerular influx of macrophages is decreased in response to sodium restriction and ACEi.\textsuperscript{7} In contrast, a high sodium diet elicited a marked blood pressure-independent pro-fibrotic response in both heart and kidneys.\textsuperscript{13} Dietary sodium was reported to have adverse effects on vascular function as well,\textsuperscript{33,34} and sodium restriction reversed the age- and hypertension-associated endothelial dysfunction, irrespective of blood pressure response.\textsuperscript{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 in 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

Figure 2. Scatter plots showing the univariate correlations between changes in creatinine clearance and changes in plasma AcSDKP induced by dietary sodium restriction on top of treatment with ACEi (A), and ACEi + ARB (B) in CKD patients.

Abbreviations: AcSDKP: n-acetyl-seryl-aspartyl-lysyl-proline, ACEi: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker.
ACE activity upon sodium restriction, 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-4, 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. 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, and interestingly, sodium restriction was found to reduce this oxidative stress. 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 modeling 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, and most likely reflects a reversible reduction in intraglomerular pressure. In our trial, indeed, it was reversible upon discontinuation of the sodium restriction. On the long-term, the short-term decrease in renal function at onset of therapy predicts a better renal outcome, attributed to lower glomerular pressure. 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 favorable 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: e.g. anti-inflammatory cytokine adiponectin was found to be inversely associated with renal function.

Dual blockade may have detrimental effects (for instance as observed in the ONTARGET trial and the recently published ALTITUDE trial). In our trial, addition of ARB to ACEi was not associated with an increase in plasma AcSDKP levels. This was as expected considering 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 had only a small effect on blood pressure, which is in line with previous meta-analysis, possible due to an inappropriate rise in circulating renin levels.

This study has several limitations. First, all liabilities associated with post-hoc studies apply. Second, this study should be considered as hypothesis-generating as the exact mechanism on how sodium restriction effects 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 subjects without renal impairment nor in subjects 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. Furthermore, ACEi is known to increase levels of AcSDKP 4 to 5 fold compared to baseline. 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 down-stream effects of AcSDKP by studying its relation to circulating fibrotic and inflammation markers, however, these data are 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 Caucasian patients with non-diabetic CKD, we found circulating levels of AcSDKP significantly and independently increased in response to sodium restriction on top of single and dual RAAS blockade. We postulate that up-regulation of AcSDKP might contribute to the enhanced renoprotective efficacy of RAAS blockade during moderate sodium restriction.

Disclosures
None to declare.

Acknowledgements
This trial was performed by the HONEST (Holland NEphrology STudy) Group.
REFERENCES


