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Low salt diet and weight loss have additive effects on the anti-proteinuric effects of angiotensin II receptor blockers in hypertensive patients with chronic kidney disease

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Abstract

Low salt diet and weight loss have additive effects on the anti-proteinuric effects of angiotensin II receptor blockers in hypertensive patients with chronic kidney disease

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Background

Efforts to delay progression to end stage renal disease (ESRD) are mainly based on the control of hypertension and diabetes. However, chronic kidney disease (CKD) is a complex and life-long disease, and physicians should integrate pharmacological and non-pharmacological therapies for the management of CKD.

Objectives

Investigator explore the additive anti-proteinuric effects of reduced salt intake and weight on the usage of an angiotensin II receptor blocker and the potential mechanisms of the beneficial effects in hypertensive CKD patients.
Methods

This study is a subanalysis of data from an open-label, randomized, controlled clinical trial (NCT01552954). Among the 235 participants, the body weight of 227 participants was measured and 24h urine samples were collected at baseline and after 16 weeks. The participants were assigned to each subgroup according to changes in their salt intake and body weight. Urinary cytokines as well as urine creatinine, sodium, urea nitrogen, and albumin excretion were measured over the 16-week study period.

Results

Adherence to ARB treatment over the 16 weeks was 95% (71.5 - 100%). The mean 24h urinary sodium and albumin excretions at enrollment were 170 ± 74 mEq/day and 1041 ± 1094 mg/day, respectively. Over the study period, a low salt diet and unintentional weight loss independently increased the probability of reduced albuminuria (low salt group: with a ≥ 25% decrease in the estimated urine sodium excretion rate after 16 weeks, RR 3.583, 95% CI 1.448 - 8.865, p=0.006; weight reduction group: with a ≥ 1.5% decrease in body weight after 16 weeks, RR 6.234, 95% CI 1.913 - 20.315, p=0.002). The relationship between weight loss and a decrease in albuminuria was even more significant in several subgroups, including participants who were female, younger (< 65 yrs), non-obese and obese (BMI ≥ 18.5 kg/m²), as well as those who had a CKD stage ≥ 3a (≥ 45 ml/min/1.73m²), consumed a low salt diet (urinary sodium excretion < 200 mEq/day), consumed a low protein diet (< 1.2 g/kg/day), and had a low baseline level of albuminuria (< 2000 mg/day). Among the urinary
cytokines, angiotensinogen was significantly decreased in the participants who reduced their salt intake and podocalyxin was noticeably decreased in the participants who lost weight (p=0.017 and p=0.013, respectively).

Conclusions

Investigator observed that the low salt diet and unintentional weight loss have additive effects on the anti-proteinuric effects of treatment with ARBs in hypertensive CKD patients, which are possibly related to the reduced intrarenal renin-angiotensin-aldosteron activation and damaged podocytes, respectively.

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keywords: low salt diet, weight loss, albuminuria, angitensin II receptor blocker, chronic kidney disease, hypertension

Student Number: 2014-30681
Contents

Abstract............................................................................................................i

Contents..........................................................................................................iv

List of Tables and Figures Legends...............................................................v

Introduction......................................................................................................1

Methods............................................................................................................3

Results..............................................................................................................9

Discussion........................................................................................................22

Reference.......................................................................................................27

Abstract in Korean........................................................................................31
List of Tables and Figure Legends

Table 1. Baseline characteristics of the participants included in this study

Table 2. The probability of a decrease in albuminuria over 16 weeks

Table 3. The percent change in the parameters over 16 weeks according to the change in body weight

Table 4. The probability of a decrease in albuminuria over 16 weeks

Table 5. The rate of achieving a reduction in albuminuria among subgroups according to the baseline characteristics

Figure 1. Study flow chart

Figure 2. Decreases in albuminuria according to the change in salt intake over 16 weeks

Figure 3. The changes in urinary cytokines according to the changes in salt intake over 16 weeks

Figure 4. Reduced albuminuria according to the change in body weight over 16 weeks

Figure 5. The changes in urinary cytokines according to the changes in body weight over 16 weeks
Introduction

Chronic kidney disease (CKD) is a well-known risk factor for end stage renal disease (ESRD), hospitalization, cardiovascular morbidity and mortality, and general mortality[1, 2]. Because the incidence and prevalence of diabetes and hypertension, which are the leading causes of CKD, have increased consistently[3, 4], CKD is a common disease worldwide[5, 6]. However, in recent years, the prevalence of CKD has plateaued in the USA[6] and decreased in the UK[7], which may be due to improvements in strategies for CKD management.

Several strategies have been established for the clinical care of CKD patients including the control of blood pressure (BP), the reduction of proteinuria through the use of renin-angiotensin system (RAS) blockers, the correction of hyperglycemia and dyslipidemia, the avoidance of nephrotoxic drugs, and lifestyle modifications such as a low salt diet, protein restriction, and weight reduction[8, 9]. Because CKD is a representative example of a highly complex and life-long disease, physicians should integrate both pharmacological and non-pharmacological therapies for the management of CKD.

The clinical trial that this study is based on was an open-label, case-controlled, randomized study exploring the proteinuria-lowering effects of a low salt diet in nondiabetic, hypertensive CKD patients who were taking RAS blockers[10]. Through this trial, the authors demonstrated that the reduction in the 24-hour urinary albumin excretion amount achieved with olmesartan therapy could be further enhanced with an intensive low salt diet education. Investigator conducted a subanalysis of the data from this trial to evaluate the additive anti-proteinuric effects of the reductions
in salt intake and weight on the usage of an angiotensin II receptor blocker and the potential mechanisms of the additive effects, in hypertensive CKD patients.
Methods

Study population

This study involved a subsequent analysis of the data from an open-label, case-controlled, randomized clinical trial (NCT01552954)[10]. The patients were selected from the outpatient renal clinics of 7 centers in Korea between March 2012 and March 2013. All participants fulfilled the following inclusion criteria: between 19 and 75 years of age; use of antihypertensive medication or a diagnosis of hypertension; a Modification of Diet in Renal Disease (MDRD)-estimated glomerular filtration rate (GFR) ≥ 30 ml/min/1.73m²; a random urine albumin to creatinine ratio ≥ 30 mg/g Cr in the last 6 months; and the ability and willingness to provide written informed consent. The exclusion criteria were as follows: uncontrolled hypertension (BP > 160/100 mmHg) at the time of screening; pregnancy; serum potassium > 5.5 mEq/L; malignancy; a diagnosis of cardiovascular disease (cerebral infarction, hemorrhagic infarction, acute myocardial infarction or unstable angina, coronary angioplasty, or coronary artery bypass surgery) within the last 6 months; a contraindication to angiotensin II receptor blockers (ARBs); diabetes mellitus; and the use of steroids or other immunosuppressive agents at the time of registration.

The sample size was calculated according to a reference from a specific article with proteinuria as an outcome[11]. A two-sided 5% significance level, a power of 80%, and a sample size of 135 patients per group were necessary given an anticipated dropout rate of 20%. To recruit this number of patients, a 12-month inclusion period was anticipated. During the study period, a total of 312 patients were screened, 269 were enrolled, 34 dropped out, and 235
completed the trial (Figure 1). Among the 235 participants, 227 provided body weight and 24h urine samples at baseline and after 16 weeks.

Figure 1. Study flow chart

Study protocol

The patients were screened 8 weeks prior to the commencement of the study (at -8 weeks), and the protocol included a “run-in period” for the adjustment of antihypertensive medications. All
participants were instructed to stop any RAS blocking agents or diuretic therapies and to switch to antihypertensive agents of different categories during this period. After an 8-week run-in period (0-week), the investigators conducted baseline laboratory investigations. From the 0-week time point, all of the enrolled patients were prescribed olmesartan medoxomil (Daewoong Pharmaceutical Co./Daiichi Sankyo Korea Co., Seoul, South Korea) at a 40 mg once-a-day fixed dose until the end of the study. After 16 weeks (16-week), the participants again underwent a laboratory investigation. At the 0- and 16-week time points, the patients were asked to collect 24hour urine samples the day before each visit to assess albuminuria, as well as urinary sodium, urea nitrogen, and creatinine excretion. Some of the urine samples from the 24hour urine collection were stored in a refrigerator at -70°C for future measurements of urine cytokine levels. Compliance was assessed using the 24hour urine samples with correction by calculating the predicted daily creatinine excretion (men: \(-12.63 \times \text{age} + 15.12 \times \text{body weight} + 7.39 \times \text{height} - 79.9 \ \text{mg/day}; \) women: \(-4.72 \times \text{age} + 8.58 \times \text{body weight} + 5.09 \times \text{height} - 74.5 \ \text{mg/day}\))[12]. The complete collection of 24hour urine was defined as [measured 24hour urine creatinine (mg/day)/21 \times \text{body weight (kg)}] \geq 0.7 by using Knuiman’s criterion [13]. Participants with poor medication adherence to olmesartan (used \(\leq 60\% \) of the prescribed medication) were removed from the study. During the 16-week period, no medications were changed except for antihypertensive medications other than olmesartan medoxomil to adjust and maintain blood pressure at 130/80 mmHg. Safety assessments included adverse events, self-reported hypotension, and select hematological and biochemical measures.
Outcome measurement

Investigator explored the additive anti-proteinuric effects of reductions in salt intake and body weight on the usage of an angiotensin II receptor blocker and the potential mechanisms of the additive effects in hypertensive CKD patients.

Dietary assessment and weight measurement

Changes in salt intake were assessed by estimating the urine sodium excretion in a day. The urine marker-to-creatinine ratio (a unit/g creatinine) was multiplied by calculating the estimated creatinine excretion rate (eCER) (g/24h) to obtain the estimated urine excretion of a marker (in a unit/24h) [14]. The equations suggested by Tanaka et al. were used to calculate the eCER. Protein intake was calculated as follows: \[24h \text{ urine urea nitrogen } \{g/\text{day}\} + (\text{body weight } \{\text{kg}\} \times 0.031 \{g \text{ nitrogen/kg/day}\}) \times 6.25 \text{ (g protein/day)}\] and normalized to body weight (g protein/kg body weight/day). At the 0- and 16-week time points, the participants’ body weights were measured using a scale. The participants were assigned to the following subgroups according to the changes in their salt intake and body weight during the 16 weeks: for the ratio of estimated urine sodium to creatinine at week 16 compared to week 0: 1) group 1, patients with a \(\geq -25\%\) decrease, decreased group; 2) group 2, patients with a \(-25 \sim -25\%\), unchanged group; and 3) group 3, patients with a \(\geq 25\%\) increase, increased group; additionally, for the ratio of the body weight at week 16 compared to week 0: 1) group 1, patients with a \(\geq 1.5\%\) decrease; 2) group 2, patients with a \(1.5\% \sim 0.1\%\) decrease; and 3) group 3, patients with a \(\geq 0.0\%\) increase.
Cytokine measurement

Several urinary cytokines including angiotensinogen (AGT), malondialdehyde (MDA), monocyte chemoattractant protein-1 (MCP-1), adiponectin, and podocalyxin were measured. Urinary concentrations of AGT were measured with a Human AGT ELISA Kit (Cusabio Biotech, Cat No. CSB-E08564h), MDA was measured with MDA assay kits (Cell Biolab, Cat No.:STA-330), MCP-1 was measured with a Human MCP-1 ELISA Kit (Quantikine kit, R&D Systems, Abingdon, UK), adiponectin was measured with a highly sensitive ELISA (BioVendor, Brno, Czech Republic), and podocalyxin was measured with human ELISA kits (Cusabio Biotech, Cat No.CSB-E09891h), according to the manufacturers’ protocols. All cytokine concentrations were measured in duplicate and adjusted for urinary creatinine concentration.

Ethics

The protocol was approved by the Institutional Review Board (IRB) of Seoul National University Bundang Hospital (IRB no. B-1112-142-008). The study procedures were in accordance with the ethical standards of the IRB of Seoul National University Bundang Hospital and the 2008 Helsinki Declaration. Written informed consent was obtained from each participant prior to inclusion.

Acknowledgement
This study was funded by Seoul National University Bundang Hospital (No. 02-2015-046).

**Statistical analysis**

Differences in the parameters between the 16- and 0-week values were calculated, as well as the percentage change \([(\text{value at 16-week} - \text{value at 0-week}) \times 100/\text{value at 0-week} \,(\%)]\). The decrease in albuminuria was defined based on the ratio of estimated albuminuria at the 16-week time point compared with that at the 0-week time point and was \(\leq -25\%\).

All analyses and calculations were performed using SPSS Statistics V21.0 (IBM Corporation, Armonk, NY, USA). Continuous variables were expressed as the mean \(\pm\) standard deviation (SD) or as a percentage for categorical variables. The Kruskal–Wallis test was used to compare continuous variables between the groups, and the Pearson’s Chi-square test or linear by linear chi-square statistic was used to analyze associations between categorical variables. The relationships between the variables were estimated using Pearson’s correlation coefficient and tested using multiple linear regression analysis for continuous variables and multiple logistic regression analysis for dichotomized variables by adjusting for related factors. Two-tailed values of \(P < 0.05\) were considered statistically significant.
Results

Baseline characteristics of participants

The clinical and laboratory findings of the study population at enrollment are presented in Table 1. The mean age was 50.3 ± 13.0 years, and 50% of the participants were male. The mean BMI value was 25.4 ± 3.8 kg/m², and 53.7% of patients were obese (BMI ≥ 25 kg/m²). The mean serum creatinine was 1.15 ± 0.41 mg/dL, and the eGFR from the MDRD equation was 67.0 ± 23.9 mL/min/1.73m². The percentage of compliance to ARB treatment over the 16 weeks was 95% (71.5 - 100%). The mean 24h urinary sodium and albumin excretion values were 170 ± 74 mEq/day and 1041 ± 1094 mg/day, respectively. Complete 24h urine collection was achieved in 76.7% of the participants, and the mean urine volume was 1.00 ± 0.06 L/day.

Table 1. Baseline characteristics of the participants included in this study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.1 ± 13.0</td>
<td>Hemoglobin (g/dL)</td>
<td>13.9 ± 1.8</td>
</tr>
<tr>
<td>Female (%)</td>
<td>114 (50.2%)</td>
<td>Creatinine (mg/dL)</td>
<td>1.15 ± 0.41</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.4 ± 3.8</td>
<td>eGFR (ml/min/1.73m²)</td>
<td>67.0 ± 23.9</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>27 (11.9%)</td>
<td>Total cholesterol (mg/dL)</td>
<td>183 ± 35</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td>Estimated 24h urine parameters</td>
<td></td>
</tr>
<tr>
<td>AMI (%)</td>
<td>1 (0.4)</td>
<td>eUcr (mg/day)</td>
<td>1305 ± 318</td>
</tr>
<tr>
<td>Angina (%)</td>
<td>1 (0.4)</td>
<td>eUalb (mg/day)</td>
<td>1041 ± 1094</td>
</tr>
<tr>
<td>CVA (%)</td>
<td>6 (2.6)</td>
<td>eUna (mEq/day)</td>
<td>170 ± 74</td>
</tr>
<tr>
<td>Number of anti-HTN</td>
<td>1.6 ± 0.9</td>
<td>eUUN (g/day)</td>
<td>7.6 ± 4.8</td>
</tr>
<tr>
<td>CCB (%)</td>
<td>213 (93.8%)</td>
<td>eAGT (ng/day)</td>
<td>517.5 ± 677.7</td>
</tr>
<tr>
<td>BB (%)</td>
<td>80 (35.2%)</td>
<td>eMCP-1 (ng/day)</td>
<td>279.9 ± 318.7</td>
</tr>
<tr>
<td></td>
<td>Others (%)</td>
<td>Drug compliance (%)</td>
<td>eMDA (μM/day)</td>
</tr>
<tr>
<td>----------------</td>
<td>------------</td>
<td>---------------------</td>
<td>---------------</td>
</tr>
<tr>
<td></td>
<td>38 (16.7)</td>
<td>95 (71.5-100)</td>
<td>2.5 ± 2.6</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>131 ± 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79 ± 9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* AMI, angina, and CVA, acute myocardial infarction, angina pectoris, and cerebrovascular accident of a thromboembolic or hemorrhagic nature, respectively, diagnosed by a physician; Number of anti-HTN, number of antihypertensive medications; Renin-angiotensin-aldosterone system blockers and diuretics were prohibited beginning at enrollment and for 8 weeks thereafter; CCB, calcium channel blocker; BB, beta-blocker; Others, other anti-HTN, such as blockers of the α-adrenergic system and vasodilators; SBP, systolic blood pressure; DBP, diastolic blood pressure; Estimated 24h urine parameters, obtained by using the estimated daily creatinine excretion from the Tanaka equation; UUN, urine urea nitrogen; Protein intake was calculated as follows: [24h urine urea nitrogen (g/day) + (body weight (kg) × 0.031 (g nitrogen/kg/day)) × 6.25 (g protein/day)] and normalized by body weight (g protein/kg body weight/day).

The change in salt intake and decreases in albuminuria

Over the study period of 16 weeks, 76.7% (174/227) of participants had an albuminuria reduction of more than 25% after treatment with ARB. The mean value of the difference in estimated albuminuria between the 16- and 0-week time points was -538.7 ± 824.1 mg/day, and the mean percentage change was -36.3 ± 130.6%. The participants were assigned to three groups according to the change in their salt intake during the 16 weeks. Eighty-one patients (36.3%) were assigned to group 1 (a ≥ -25% change in the ratio of estimated urine sodium to creatinine, decreased group), 89 patients (39.9%) were assigned to group 2 (a -25 ~ 25% change in the ratio of estimated urine sodium to creatinine,
unchanged group), and 53 patients (23.8%) were assigned to group 3 (a \( \geq 25\% \) change in the ratio of estimated urine sodium to creatinine, increased group).

The difference in estimated albuminuria between the 16- and 0-week time points was compared among the three groups using the Kruskal-Wallis test (Figure 2-A). With more participants who had a low salt diet over the period, there was a greater difference in estimated albuminuria. The percentage of participants who achieved a > 25% decrease in albuminuria 16 weeks after treatment with ARB compared with baseline was 86.4% in group 1, 76.4% in group 2, and 67.9% in group 3 (p=0.010, Figure 2-B). A multiple logistic regression analysis was used to evaluate the probability of a decrease in estimated albuminuria during 16 weeks that was adjusted for age, sex, and other factors related to the decrease in estimated albuminuria, such as albuminuria at baseline, percent changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), total CO\(_2\), and serum total cholesterol (Table 2). As shown in Table 2, the participants of group 1 (a \( \geq -25\% \) change in the ratio of estimated urine sodium) had the highest probability of a decrease in estimated albuminuria.
Figure 2. Decreases in albuminuria according to the change in salt intake over 16 weeks

(A) The difference in estimated albuminuria between 0 and 16 weeks, (B) The percentage of participants who achieved a > 25% decrease in albuminuria. Group 1 (a ≥ -25% change in the ratio of estimated urine sodium, decreased group), group 2 (a -25 ~ 25% change in the ratio of estimated urine sodium, unchanged group), and group 3 (a ≥ 25% change in the ratio of estimated urine sodium, increased group). The bar represents the 95% confidence interval of the mean value. *P-values were estimated by Kruskal-Wallis test. **P-value estimated by Pearson’s Chi-square test.

Table 2. The probability of a decrease in albuminuria over 16 weeks

<table>
<thead>
<tr>
<th>Percent change in SBP (%)</th>
<th>B</th>
<th>Wald</th>
<th>RR</th>
<th>95% CI of RR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.050</td>
<td>9.879</td>
<td>0.951</td>
<td>0.922 - 0.981</td>
<td>0.020</td>
</tr>
<tr>
<td>Percent change in T-Chol. (%)</td>
<td>-0.08</td>
<td>3.777</td>
<td>0.982</td>
<td>0.964 - 1.000</td>
<td>0.052</td>
</tr>
<tr>
<td>Change in salt intake group</td>
<td>7.661</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>1.276</td>
<td>7.621</td>
<td>3.583</td>
<td>1.448 - 8.865</td>
<td>0.006</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.507</td>
<td>1.552</td>
<td>1.661</td>
<td>0.748 - 3.690</td>
<td>0.213</td>
</tr>
</tbody>
</table>

RR: relative risk, SBP: systolic blood pressure, T-Chol: total cholesterol, Change in salt intake group: compares the relative risk of achieving
decrease in albuminuria to Group 3. Group 1 (a ≥ -25% change in the ratio of estimated urine sodium to creatinine during 16 weeks, decreased group), group 2 (a -25 ~ 25% change in the ratio of estimated urine sodium to creatinine during 16 weeks, unchanged group), and group 3 (a ≥ 25% change in the ratio of estimated urine sodium to creatinine during 16 weeks, increased group). The P-values were estimated by multiple logistic regression analysis adjusted with age, gender and the related factors to achieve a decrease in albuminuria, such as albuminuria at baseline, percent changes in SBP, DBP, total CO₂, serum total cholesterol, and change in salt intake group.

Next, the frequencies of decreases in the following urinary cytokines over the 16 weeks were evaluated according to the change in salt intake: AGT, MCP-1, MDA, adiponectin, and podocalyxin (Figure 3). Only for angiotensinogen, which is a marker of intrarenal RAS activity, was the frequency of decrease statistically significant across the three groups (P=0.017).
Figure 3. The changes in urinary cytokines according to the changes in salt intake over 16 weeks

*Frequency of reduced cytokine levels: The frequency of a 25% or more reduction in the 24-hour urinary cytokine to creatinine ratio at 16 weeks compared to 0 weeks 25%. Group 1 (a ≥ -25% change in the ratio of estimated urine sodium to creatinine, decreased group), group 2 (a -25 ~ 25% change in the ratio of estimated urine sodium to creatinine, unchanged group), and group 3 (a ≥ 25% change in the ratio of estimated urine sodium to creatinine, increased group). AGT: angiotensinogen, MCP1: monocyte chemoattractant protein-1, MDA: malondialdehyde, APN: adiponectin, PCX: podocalyxin.

The change in body weight and decreases in albuminuria

The participants were divided into three groups according to the change in their body weight over the 16 weeks. Fifty-eight participants (25.7%) were assigned to group 1 (a ≥ 1.5% decrease in body weight after 16 weeks), 32 participants (14.2%) were assigned to group 2 (a 1.5% ~ 0.1% decrease in body weight), and 136 participants (60.2%) were assigned to group 3 (a ≥ 0.0% increase in body weight). The mean differences in weight over the period were -2.41 ± 1.45 kg in group 1, -0.55 ± 0.33 kg in group 2, and 1.28 ± 1.74 kg in group 3 (Table 3). The percent changes in the clinical parameters during the 16 weeks were also evaluated according to the change in body weight (Table 3). The ratios of estimated protein intake, SBP, total cholesterol, and eGFR between the 16- and 0-week time points did not significantly differ among the three groups. However, the percent change in urinary sodium excretion over the 16 weeks was significantly different among the three groups (p=0.017).
Table 3. The percent change in the parameters over 16 weeks according to the change in body weight

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>58</td>
<td>32</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td>Difference in BWt (kg)</td>
<td>-2.41 ± 1.45</td>
<td>-0.55 ± 0.33</td>
<td>1.28 ± 1.74</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ratio of 24h uNa/Cr (%)*</td>
<td>-15.2 ± 37.5</td>
<td>-5.3 ± 40.2</td>
<td>6.1 ± 52.7</td>
<td>0.017</td>
</tr>
<tr>
<td>Ratio of protein intake (%)*</td>
<td>9.8 ± 58.5</td>
<td>18.9 ± 67.4</td>
<td>20.0 ± 75.5</td>
<td>0.988</td>
</tr>
<tr>
<td>Ratio of SBP (%)*</td>
<td>-6.1 ± 12.5</td>
<td>-10.2 ± 7.9</td>
<td>-6.6 ± 10.9</td>
<td>0.232</td>
</tr>
<tr>
<td>Ratio of T-Chol (%)*</td>
<td>-7.9 ± 14.9</td>
<td>-3.4 ± 14.2</td>
<td>-1.7 ± 19.2</td>
<td>0.052</td>
</tr>
<tr>
<td>Ratio of estimated GFR (%)*</td>
<td>-6.1 ± 14.6</td>
<td>-3.7 ± 13.3</td>
<td>-4.8 ± 13.6</td>
<td>0.633</td>
</tr>
</tbody>
</table>

*Group 1: patients with a decrease in body weight ≥ 1.5% during 16 weeks, Group 2: patients with a decrease in body weight of 1.5% ~ 0.1% during 16 weeks, Group 3: patients with an increase in body weight ≥ 0.0% during 16 weeks, BWt: body weight, SBP: systolic blood pressure, T-Chol: total cholesterol. The difference in BWt was calculated as follows: BWt at 16-week - BWt at 0-week. The ratio of parameters; 24h uNa/Cr (estimated 24hr urine sodium to creatinine ratio, mEq/g cr), protein intake (g/kg/day), SBP (mmHg), total cholesterol (mg/dL), estimated GFR (ml/min/1.73m²) were calculated, as well as the percentage change [value at 16-week - value at 0-week) × 100/value at 0-week (%)].

The percent change in estimated albuminuria over the 16 weeks was compared among the three groups using the Kruskal–Wallis test (Figure 4-A). With a greater decrease in body weight over the 16 weeks, there was a greater reduction in the estimated albuminuria. The percentage of participants who achieved a > 25% decrease in estimated albuminuria after treatment with ARB compared with baseline was 93.0% in group 1, 80.6% in group 2, and 71.1% in group 3.
A multiple logistic regression analysis was also used to evaluate the probability of a decrease in the estimated albuminuria after 16 weeks adjusting for age, sex, and other factors related to a decrease in estimated albuminuria levels, such as albuminuria at baseline, percent changes in SBP, DBP, eGFR, total CO2, serum total cholesterol, and 24h urine excretion of sodium (Table 4). As shown in Table 4, the probability of a decrease in estimated albuminuria with ARB treatment was higher in the study population with baseline albuminuria and reduced body weight after 16 weeks. In particular, the participants of group 1 (with a ≥ 1.5% decrease in body weight after 16 weeks) showed the highest probability of a decrease in the estimated albuminuria levels. The interactions were explored between changes in body weight and other independent factors, such as baseline albuminuria, change in SBP, estimated urinary sodium excretion, and estimated protein intake over 16 weeks. There were no interactions between the changes in body weight and the other independent factors.

Figure 4. Reduced albuminuria according to the change in body weight over 16 weeks
The percent change in albuminuria over 16 weeks, The percentage of participants who achieved a > 25% decrease in albuminuria. Group 1 (a ≥ 1.5% decrease in body weight), group 2 (a 1.5 ~ 0.1% decrease in body weight), and group 3 (a ≥ 0.0% increase in body weight). The bar represents the 95% confidence interval of the mean value. *P-values were estimated by Kruskal–Wallis test. **P-value estimated by Pearson’s Chi-square test.

Table 4. The probability of a decrease in albuminuria over 16 weeks

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Wald</th>
<th>RR</th>
<th>95% CI of RR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albuminuria at baseline (mg/day)</td>
<td>0.212</td>
<td>4.781</td>
<td>1.236</td>
<td>1.022 - 1.494</td>
<td>0.029</td>
</tr>
<tr>
<td>Percent change in SBP (%)</td>
<td>-0.062</td>
<td>13.237</td>
<td>0.940</td>
<td>0.910 - 0.972</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percent change in 24h uNa (%)</td>
<td>-0.009</td>
<td>6.235</td>
<td>0.991</td>
<td>0.984 - 0.998</td>
<td>0.013</td>
</tr>
<tr>
<td>Change in body weight group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>1.830</td>
<td>9.217</td>
<td>6.234</td>
<td>1.913 - 20.315</td>
<td>0.002</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.188</td>
<td>0.133</td>
<td>1.207</td>
<td>0.438 - 3.326</td>
<td>0.716</td>
</tr>
</tbody>
</table>

RR: relative risk, Albuminuria at baseline: estimated 24h albumin excretion, obtained by using the estimated daily creatinine excretion from the Tanaka equation. SBP: systolic blood pressure, 24h uNa: 24h urine sodium was estimated 24h sodium excretion, obtained by using the estimated daily creatinine excretion from the Tanaka equation. Change in body weight group: compares the relative risk of achieving a decrease in albuminuria to Group 3. Group 1: patients with a decrease in body weight ≥ 1.5% during 16 weeks, Group 2: patients with a decrease in body weight of 1.5% ~ 0.1% during 16 weeks, Group 3: patients with an increase in body weight ≥ 0.0% during 16 weeks. The P-values were estimated by multiple logistic regression analysis adjusted with age, gender, season at enrollment, and the related factors to achieve a decrease in albuminuria, such as albuminuria at baseline, percent changes in SBP, DBP, eGFR, total CO₂, serum total cholesterol, 24h urine excretion of sodium, and change in body weight group.
Next, correlations between the frequencies of reduced cytokines and changes in weight were assessed. The participants with reduced body weight had a higher frequency of decreases in the following urinary cytokines after 16 weeks: AGT, MCP-1, MDA, adiponectin, and podocalyxin (Figure 5). However, only the decrease in podocalyxin, as a marker of podocyte injury, was statistically significant across the groups (p=0.013).

![Figure 5. The changes in urinary cytokines according to the changes in body weight over 16 weeks](image)

*Frequency of decrease in cytokine level: The frequency of a 25% or greater reduction in the 24hour urinary cytokine to creatinine ratio at 16 weeks compared to 0 week. Group 1 (a ≥ 1.5% decrease in body weight), group 2 (a 1.5 ~ 0.1% decrease in body weight), and group 3 (a ≥ 0.0% increase in body weight)

Finally, subsequent analyses regarding the weight change and decreased albuminuria were conducted according to baseline characteristics such as gender (female vs. male), age (< 65 years vs. ≥ 65 years), BMI (18.5 ≤ BMI < 25 kg/m² vs. BMI ≥ 25 kg/m²), eGFR (≥ 45 ml/min/1.73m² vs. < 45 ml/min/1.73m²), urinary sodium excretion (< 200 mEq/day vs. ≥ 200 mEq/day), protein intake (< 1.2 g/kg/day vs. ≥ 1.2 g/kg/day), and albuminuria (< 2000 mg/day vs. ≥ 2000 mg/day) (Table 5). The proportion of participants who achieved a decrease in albuminuria of > 25% at 16 weeks increased according to changes in body weight during the 16-week study period. In particular, these trends were significant in participants who were female, younger (< 65 years), non-obese and obese (BMI ≥ 18.5 kg/m²), with a CKD stage ≥ 3a (≥ 45 ml/min/1.73m²), who consumed a low salt diet (urinary sodium excretion < 200 mEq/day), or a low protein diet (< 1.2 g/kg/day), and with a low baseline level of albuminuria (< 2000 mg/day).
Table 5. The rate of achieving a reduction in albuminuria among subgroups according to the baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>91.2%</td>
<td>81.8%</td>
<td>66.2%</td>
<td>0.019</td>
</tr>
<tr>
<td>Men</td>
<td>95.7%</td>
<td>80.0%</td>
<td>76.1%</td>
<td>0.120</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 65</td>
<td>91.7%</td>
<td>82.1%</td>
<td>73.9%</td>
<td>0.036</td>
</tr>
<tr>
<td>≥ 65</td>
<td>100.0%</td>
<td>66.7%</td>
<td>58.3%</td>
<td>0.068</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.5 ≤ BMI &lt; 25</td>
<td>100.0%</td>
<td>75.0%</td>
<td>69.1%</td>
<td>0.027</td>
</tr>
<tr>
<td>≥ 25.0</td>
<td>91.4%</td>
<td>88.9%</td>
<td>71.2%</td>
<td>0.032</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 45</td>
<td>91.5%</td>
<td>78.3%</td>
<td>71.0%</td>
<td>0.020</td>
</tr>
<tr>
<td>&lt; 45</td>
<td>100.0%</td>
<td>87.5%</td>
<td>71.4%</td>
<td>0.127</td>
</tr>
<tr>
<td>Sodium excretion (mEq/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 200</td>
<td>95.0%</td>
<td>83.3%</td>
<td>73.7%</td>
<td>0.016</td>
</tr>
<tr>
<td>≥ 200</td>
<td>88.2%</td>
<td>71.4%</td>
<td>63.9%</td>
<td>0.165</td>
</tr>
<tr>
<td>Protein intake (g/kg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1.2</td>
<td>91.7%</td>
<td>76.9%</td>
<td>69.4%</td>
<td>0.010</td>
</tr>
<tr>
<td>≥ 1.2</td>
<td>100.0%</td>
<td>100.0%</td>
<td>82.6%</td>
<td>0.308</td>
</tr>
<tr>
<td>Albuminuria (mg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2000</td>
<td>91.1%</td>
<td>76.9%</td>
<td>68.7%</td>
<td>0.012</td>
</tr>
<tr>
<td>≥ 2000</td>
<td>100.0%</td>
<td>100.0%</td>
<td>85.0%</td>
<td>0.250</td>
</tr>
</tbody>
</table>

*Group 1: patients with a decrease in body weight ≥ 1.5% over 16 weeks, Group 2: patients with a decrease in body weight of 1.5% ~ 0.1% over 16 weeks, and Group 3: patients with an increase in body weight ≥ 0.0% over 16 weeks. Sodium excretion and albuminuria: Estimated 24h urine parameters, obtained using the estimated daily creatinine excretion from the Tanaka equation. Protein intake was calculated as follows: [24h urine urea nitrogen
\{(\text{g/day}) + (\text{body weight (kg} \times 0.031 (\text{g nitrogen/kg/day})) \times 6.25 (\text{g protein/day})

and normalized by body weight (g protein/kg body weight/day). P-values were calculated by Pearson’s Chi-square test.
Discussion

In this prospective clinical study, investigator demonstrated that reduced salt intake and weight reduction had additive effects on the anti-proteinuric effects of ARB in hypertensive CKD patients. Over the study period, a low salt diet and unintentional weight loss independently increased the probability of a reduction in albuminuria. Additionally, the relationship between weight loss and a decrease in albuminuria was even more significant in several subgroups including participants who were female, younger (< 65 years), non-obese and obese (BMI ≥ 18.5 kg/m²), as well as those who had a CKD stage ≥ 3a (≥ 45 ml/min/1.73m²), consumed a low salt diet (urinary sodium excretion < 200 mEq/day), consumed a low protein diet (< 1.2 g/kg/day), and had a low baseline level of albuminuria (< 2000 mg/day). Among the urinary cytokines, which were investigated to understand the mechanism of decreased albuminuria, angiotensinogen was significantly decreased in the participants who reduced their salt intake and podocalyxin was noticeably decreased in the participants who lost weight.

As mentioned above, CKD is a life-long disease and requires not only the integration of complex management measures into everyday life but also a change in lifestyle, including diet, exercise, and weight control. In this regard, CKD management should merge pharmacological and non-pharmacological therapies, such as lifestyle modification. There are several representative results of clinical studies supporting these concepts. A post hoc analysis including non-diabetic CKD patients enrolled in the REIN study showed that the anti-proteinuric effect of the RAS inhibitor was significantly higher in low salt diet (LSD) patients compared with medium-salt diet (MSD) or high salt diet (HSD), after 3 months of treatment.
The incidence of ESRD was also the highest in HSD patients (32.1%) [15]. This study also proved that decreased salt intake had an additive effect on the anti-proteinuric effects of ARB in hypertensive CKD participants. Furthermore, investigator observed that urinary angiotensinogen, as a marker of renal RAS status, was significantly reduced in proportion to the decrease in salt intake. These results suggest that the decrease in renal RAS activity induced by a low salt diet might be related to decreased albuminuria. Furthermore, this study is the first clinical study to show the relationship between a decrease in salt intake and a decrease in intra-renal RAS activity.

Many studies have explored the relationship between weight gain and the progression of CKD. Overweight and obesity are known to lead to an increase in the incidence of diabetes and hypertension, which are independent risk factors for and the leading causes of ESRD[16-19]. Recently, obesity itself has been proposed to be an independent risk factor for CKD and ESRD by several observational studies[18, 20, 21]. Consequently, lifestyle changes such as calorie- and salt-controlled diets and regular exercise, which can lead to weight reduction, have been considered to be important to the management of early-stage CKD[5, 22]. However, most of the evidence in support of these concepts was based on studies that involved obese CKD patients. The majority of these studies excluded participants with BMI < 25 kg/m². Therefore, there are very few studies that have explored the effects of weight reduction on the management of non-obese CKD patients. In this study, although weight loss had additive effects on the management of albuminuria in obese CKD patients, it had the same effects on non-obese CKD patients (Table 5).

Obesity and overweight lead to alterations in renal hemodynamics,
which are characterized by increased glomerular filtration, filtration pressure, and perfusion[23–25]. Obesity also augments sympathetic activity[26], activates the renin-angiotensin system[27, 28], activates insulin resistance[29], increases inflammation and oxidative stress[30], and attenuates the bioactivity of nitric oxide[31, 32]. Furthermore, the levels of adipose tissue–derived adipokines, which influence podocyte biology, are altered in obese patients[33]. Adiponectin is one of the adipokines that is decreased in obese patients, and its levels can be normalized by weight reduction[34]. Based on these pathophysiologies, the beneficial effects of weight reduction on renal damage induced by overweight might be due to the decreases in hyperfiltration, filtration pressure, inflammation, and oxidative stress, as well as the increases in nitric oxide bioactivity and adiponectin levels. Therefore, weight loss effectively decreases proteinuria. Among the urinary cytokines that were measured in this study, only podocalyxin significantly decreased with the percent change in body weight over 16 weeks (Figure 5). Albuminurina was also significantly decreased with the percent change in body weight (Figure 4). Through this study, a close relationship between unintentional weight loss and both albuminuria as a result of glomerular injury and podocalyxin as a marker of podocyte damage was observed. Consequently, investigator suggest that unintentional weight loss reduces albuminuria as a result of improved glomerular injury through ameliorating podocyte damage.

The reduction of proteinuria is one of the most valuable treatments for CKD patients and is caused by pharmacological and non–pharmacological management methods[35, 36]. Therefore, the results of this study, which showed additive beneficial effects of a low salt diet and unintentional weight reduction on the treatment of
albuminuria in non-obese and obese hypertensive CKD patients, are very meaningful. In particular, there is a significant additive effect of weight loss on the reduction of proteinuria, even though the weight reduction during the study period was minimal. Of course, a low salt diet and protein restriction diet as well as weight loss are also related to decreased proteinuria. However, investigator demonstrated that minimal weight reduction reduced proteinuria independently and that the decreased proteinuria induced by weight loss was related to podocyte damage.

This study also has some limitations. First, 24-hour volume urine was used and body weight was measured at a single time point for each follow-up visit. Urine excretion could be influenced by the dietary intake of patients at certain time points. Second, this study was not designed to explore the effects of dietary change and weight loss on the anti-proteinuric effects of RAS blockers. Hence, there might be confounding factors that were not considered regardless of the multivariate analysis. Third, only one parameter was used for each signal of RAS, inflammation, ROS, and podocyte injury, which reduces the accuracy of detecting the signal in the kidney. Although the urine parameters were standardized by urine creatinine levels to remove error induced by inappropriate urine collection, their accuracy could be affected by this error. Finally, urine samples were stored until the end of the study and the cytokines were measured 6 months after the first period of urine collection. During this period, the urine contents could have been altered in each sample, introducing an accuracy error.

In conclusion, investigator demonstrated that a low salt diet and unintentional weight loss have additive effects on the anti-proteinuric effects of treatment with ARBs in hypertensive CKD patients. In
particular, the beneficial effect of weight loss on reduced proteinuria is more significant in several subgroups, as mentioned above. Therefore, physicians should consider recommending a low salt diet and weight reduction for hypertensive CKD patients who are being treated with ARBs.
References


고혈압성 만성 신장병 환자에서 저염식과 체중감량이
안지오텐신 수용체차단제에 의한 단백뇨 감소 효과에
미치는 영향

배경
만성 신장병 환자에서 말기 신부전으로의 진행을 늦추는 치료는 혈압 및
혈당 조절에 근간을 두고 있다. 그러나 만성 신장병은 여러 요인들이 관
여된 복합적인 만성 질환이기 때문에, 약물학적 치료 뿐 아니라 비약물학
적 치료도 고려되어야 한다.

목적
저자는 본 연구를 통해 고혈압성 만성 신장병 환자에서 저염식과 체중감
량이 안지오텐신 수용체차단제에 의한 단백뇨 감소 효과에 미치는 영향
과 관련 기전에 대해 살펴보고자 한다.

방법
본 연구는 개방 표지, 무작위, 관리화 임상 시험연구 (NCT01552954)의
자료를 바탕으로 분석하였다. 235명의 참여자 중에서 0주제와 16주제에
체중 측정치와 24시간 수집된 소변 검체가 있는 227명을 대상으로 분석
하였으며, 이들을 16주 동안의 영분섭취 변화량과 체중의 변화량에 따라
하위 그룹으로 나누었다. 또한 소변 검체에서 싸이토카인, 크레아티닌,
소디움, 요소질소, 알부민 등을 측정하였다.
결과
16주 동안 안지오텐신 수용체차단제에 대한 평균 순응도는 95% (71.5-100%)였다. 연구 참여시 측정된 소변의 소다륨 및 일부분 배설량의 평균값은 각각 170 ± 74 mEq/day와 1041 ± 1094 mg/day였다. 연구기간동안 저염식을 시행한 군 (16주 동안 요중 소다럼 배설량이 25% 감소된 군)과 체중이 감소된 군 (16주 동안 1.5% 이상 체중이 감소된 군)은 독립적으로 단백뇨가 감소될 확률을 증가시켰다 (저염식군: RR 3.583, 95% C.I. 1.448 - 8.865, p=0.006; 체중 감소군: RR 6.234, 95% C.I. 1.913 - 20.315, p=0.002). 체중 감소와 단백뇨 감소의 관련성은 여성, 65세 미만군, 정상 체중군과 비만군, 만성 신장병 3a 이상군 (사구체여과율 ≥ 45 ml/min/1.73m2), 연구 참여 당시 저염식 (요중 소다럼 배설량 < 200 mEq/day), 및 저단백식 (< 1.2 g/kg/day)을 섭취하던 군, 단백뇨가 적은 군 (요 알부민 < 2000 mg/day)에서 더 뚜렷하게 나타났다. 소변 검체에서 측정한 싱아토카인 중, 16주동안 염분 섭취가 감소된 군에서 요중 안지오텐신시노겐 (angiotensinogen)이 유의하게 감소되었고 (p=0.017), 체중이 감소된 군에서 요중 podocalyxin이 유의하게 감소되었다 (p=0.013).

결론
저자는 본 연구를 통해 고혈압성 만성 신장병 환자에서 저염식과 체중감소가 안지오텐신 수용체차단제에 의한 단백뇨 감소에 미치는 상승적인 효과를 관찰 할 수 있었으며, 그 기전에는 신장내 레닌-안지오텐신-알도스테론 활성도의 감소와 문어발세포 (podocyte) 손상의 감소가 관련되어 있음을 보여주었다.

.................................
주요어: 저염식, 체중감소, 단백뇨, 안지오텐신 수용체차단제, 만성신장병, 고혈압
학번: 2014-30681