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KIDNEY RESEARCH AND CLINICAL PRACTICE

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Weight loss has an additive effect on the proteinuria reduction of angiotensin II receptor blockers in hypertensive patients with chronic kidney disease

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Background: Weight reduction is a lifestyle intervention that has been introduced for prevention and management of chronic kidney disease (CKD). We investigate the additive anti-proteinuric effect of weight reduction on the usage of angiotensin II receptor blockers (ARBs) and its potential mechanisms in hypertensive CKD patients.

Methods: This study is a subanalysis of data from an open-label, randomized, controlled clinical trial. Among the 235 participants, 227 were assigned to subgroups according to changes in body weight.

Results: Fifty-eight participants (25.6%) were assigned to group 1 ($\geq$ 1.5% decrease in body weight after 16 weeks), 32 participants (14.1%) were assigned to group 2 (1.5–0.1% decrease in body weight), and 136 participants (59.9%) were assigned to group 3 ($\geq$ 0.0% increase in body weight). Characteristics at enrollment were not different among the three groups, but mean differences in weight and percent changes in urinary sodium excretion over the period were statistically different ($P < 0.001$ and $P = 0.017$). Over the study period, unintentional weight loss independently increased the probability of reduced albuminuria (group 1, relative risk 6.234, 95% confidence interval 1.913–20.315, $P = 0.002$). Among urinary cytokines, only podocalyxin level decreased significantly in participants who lost weight ($P = 0.013$).

Conclusion: We observed that weight loss had an additive effect on the anti-proteinuric effects of ARBs in nondiabetic hypertensive CKD patients, although it was minimal. An additive effect was shown in both obese and non-obese participants, and its possible mechanism is related to reduction of podocyte damage.

Keywords: Angiotensin type II receptor blocker, Chronic renal insufficiency, Hypertension, Proteinuria, Weight loss

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, 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 United States [6] and decreased in the United Kingdom [7], which may be due to improved strategies for CKD management.

Several strategies have been established for clinical care of CKD patients, including blood pressure (BP) control, reduced proteinuria through the use of renin-angiotensin system (RAS) blockers, correction of hyperglycemia and dyslipidemia, avoidance of nephrotoxic drugs, and lifestyle modifications such as low salt diet and protein restriction [8,9]. Weight reduction through restricted diets, exercise therapy, anti-obesity drugs, and bariatric surgery is another commonly used lifestyle intervention. Traditionally, weight gain and obesity have contributed to the development of CKD and ESRD [10,11]. For that reason, weight reduction has been considered important for management of CKD [5,12]. However, the evidence in support of these concepts was based on studies that involved obese CKD patients. The majority of these studies excluded participants with body mass index (BMI) < 25 kg/m^2. Therefore, the clinical implications of weight loss in patients with CKD should be explored, even if patients are not obese or the degree of weight loss is minimal.

This is a subanalysis result of an open-label, case-controlled, randomized clinical trial exploring the proteinuria-lowering effects of a low salt diet in nondiabetic, hypertensive CKD patients taking RAS blockers [13]. All participants in the study were prescribed RAS blockers to control BP and reduce proteinuria and were educated about low-salt diet (LSD), although the intensity of education differed. Even if not intended, the weight of participants changed during the study period. The additive effects of unintentional weight loss on clinical progression of study participants taking RAS blockers and the potential mechanisms of those effects were evaluated.

Methods

Study population

This study involved a subsequent analysis of data from an open-label, case-controlled, randomized clinical trial (NCT01552954) [13]. The patients were selected from 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; Modification of Diet in Renal Disease (MDRD)-estimated glomerular filtration rate (eGFR) ≥ 30 mL/min/1.73 m^2; 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; 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; contraindication to angiotensin II receptor blockers (ARBs); diabetes mellitus; and the use of steroids or other immunosuppressive agents at the time of registration.
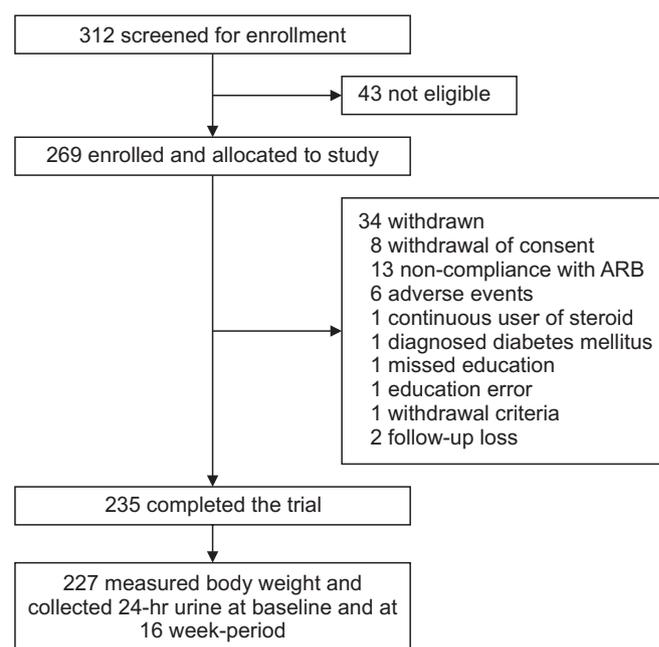
Figure 1. Study flow chart.
ARB, angiotensin II receptor blocker.

The sample size was calculated according to a reference from a specific article with proteinuria as an outcome [14]. A two-sided 5% significance level, power of 80%, and 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, 312 patients were screened, 269 patients were enrolled, 34 patients dropped out, and 235 patients completed the trial (Fig. 1). Among 235 participants, 227 provided body weight and 24-hour urine samples at baseline and after 16 weeks.

Study protocol

The patients were screened 8 weeks prior to commencement of the study. The protocol included a "run-in period" for 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 enrolled patients were prescribed olmesartan medoxomil (Daewoong Pharmaceutical Co./Daiichi Sankyo Korea Co., Seoul, Korea) at a 40 mg daily fixed dose until the end of the study. After another 8 weeks (week 8), participants were randomly assigned (1:1) to either conventional education (control) or intensive education for LSD using computerized block randomization with block size six and balanced according to institution and gender. It was not feasible to mask participants to allocation, but clinicians were masked to group assignment. Participants in the conventional education group received routine LSD education at an outpatient clinic. Participants in the intensive education group were closely supported by a dietary consultant and provided feedback by telephone for 30 minutes once a week during the study period.

After 16 weeks (16-week), participants again underwent a laboratory investigation. At the 0- and 16-week time points, patients were asked to collect 24-hour urine samples on the day before each visit to assess albuminuria, urinary sodium, urea nitrogen, and creatinine excretion (Supplementary Fig. 1; available at https://doi.org/10.23876/j.krcp.2018.37.1.49). Some urine samples from the 24-hour urine collection were stored in a re-

frigerator at −70°C for future measurements of urine cytokine levels. Compliance was assessed using the 24-hour urine samples with correction by calculating the predicted daily creatinine excretion (male: −12.63 × age + 15.12 × body weight + 7.39 × height − 79.9 mg/day; female: −4.72 × age + 8.58 × body weight + 5.09 × height − 74.5 mg/day) [15]. Participants with poor medication adherence to olmesartan (used ≤ 60% of the prescribed medication) were removed from the study. During the 16 weeks, no medications were changed, other than olmesartan medoxomil, to adjust and maintain BP at 130/80 mmHg, and none of the participants were prescribed diuretics. BP measurements were performed when patients visited the clinic. The sphygmomanometer differed depending on the institution, and the number of measurements was not confirmed. Safety assessments included adverse events, self-reported hypotension, and select hematological and biochemical measures.

Ethics approval and consent to participate

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.

Outcome measurements

The additive anti-proteinuric effects of weight reduction on usage of an ARB were analyzed, and the potential mechanisms of those effects in hypertensive CKD patients were explored.

Weight measurement

At the 0- and 16-week time points, participant body weights were measured using a scale. Participants were assigned to the following subgroups according to changes in body weight during the 16 weeks by the ratio of body weight at week 16 compared to week 0: 1) group 1, patients with ≥ 1.5% decrease; 2) group 2, patients with 1.5% to 0.1% decrease; and 3) group 3, patients with ≥ 0.0% increase.

Cytokine measurements

Several urinary cytokines including angiotensinogen (AGT), malondialdehyde (MDA), monocyte chemoattractant protein-1 (MCP-1), adiponectin, and podocalyx-in were measured. Urinary concentrations of AGT were measured with a Human AGT ELISA Kit (Cat No. CSB-E08564h; Cusabio Biotech, College Park, MD, USA), MDA was measured with MDA assay kits (Cat No. STA-330; Cell Biolab, Inc., San Diego, CA, USA), MCP-1 was measured

Table 1. Basal characteristics of patients according to change of body weight

Parameter	Group 1 (n = 58)	Group 2 (n = 32)	Group 3 (n = 136)	*P* value
Age (yr)	49.7 ± 12.4	48.2 ± 14.2	50.6 ± 12.9	0.798
Sex, female (%)	60.3	34.4	50.0	0.061
Body weight (kg)	69.0 ± 14.7	69.8 ± 9.4	67.4 ± 13.6	0.352
BMI (kg/m^2)	26.0 ± 3.9	25.5 ± 2.7	25.1 ± 3.9	0.231
Current drinker (%)	37.9	43.8	40.4	0.750
Current smoker (%)	12.1	9.4	12.5	0.886
History of AMI (%)	0.0	0.0	0.7	0.717
History of angina (%)	0.0	0.0	0.7	0.715
History of CVA (%)	3.4	0.0	2.9	0.589
Number of anti-HTNs	1.64 ± 1.00	1.44 ± 0.72	1.59 ± 0.82	0.546
CCB (%)	93.1	96.9	93.4	0.737
BB (%)	39.7	31.3	34.6	0.690
Others* (%)	24.1	6.3	16.2	0.090
SBP (mmHg)	132 ± 11	130 ± 11	132 ± 12	0.443
DBP (mmHg)	79 ± 8	81 ± 8	80 ± 10	0.374
Total cholesterol (mg/dL)	177 ± 25	183 ± 34	185 ± 39	0.620
Hemoglobin (g/dL)	13.8 ± 1.7	14.2 ± 1.9	13.9 ± 1.7	0.475
Creatinine (mg/dL)	1.11 ± 0.33	1.23 ± 0.43	1.14 ± 0.43	0.496
eGFR (mL/min/1.73 m^2)	65.6 ± 21.4	65.6 ± 24.3	67.9 ± 25.0	0.851
Estimated (e) 24-hr urine parameters[†]				
eUcr (mg/d)	1,300 ± 355	1,364 ± 233	1,294 ± 320	0.322
eUalb (mg/d)	1,102 ± 1,167	971 ± 799	1,033 ± 1,127	0.821
eUna (mEq/d)	177 ± 82	160 ± 61	167 ± 72	0.758
eUUN (g/d)	7.7 ± 4.2	7.8 ± 4.4	7.6 ± 5.2	0.680
eAGT (ng/d)	564 ± 692	508 ± 476	504 ± 718	0.340
eMCP-1 (ng/d)	267 ± 204	284 ± 206	286 ± 377	0.194
eMDA (µM/d)	2.2 ± 1.7	2.9 ± 2.9	2.5 ± 2.8	0.263
eAdiponectin (µg/d)	88.6 ± 99.1	81.4 ± 97.1	85.0 ± 133.5	0.188
ePodocalyxin (ng/d)	11.4 ± 11.1	10.0 ± 7.4	9.5 ± 9.6	0.390
Protein intake (g/kg/d)[‡]	0.92 ± 0.44	0.90 ± 0.40	0.93 ± 0.51	0.997

Data are presented as mean ± standard deviation or percent only.

Group 1, patients with decreased body weight ≥ 1.5% during 16 weeks; group 2, patients with decreased body weight of 1.5—0.1% during 16 weeks; group 3, patients with increased body weight ≥ 0.0% during 16 weeks.

AGT, angiotensinogen; AMI, acute myocardial infarction; anti-HTNs, antihypertensive medications; BB, beta-blocker; BMI, body mass index; CCB, calcium channel blocker; CVA, cerebrovascular accident; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; SBP, systolic blood pressure; Ualb, urine albumin; Ucr, urine creatinine concentration; Una, urine sodium; UUN, urine urea nitrogen.

P values for continuous variables were estimated by Kruskal—Wallis test, and *P* values for categorical variables were calculated by Pearson's chi-square test.

*Other anti-HTNs, such as α-adrenergic system blockers and vasodilators.

[†]Estimated 24-hr urine parameters were obtained using estimated daily creatinine excretion from the Tanaka equation.

[‡]Protein intake was calculated as follows: "24-hr UUN (g/day) + {body weight [18] × 0.031 (g nitrogen/kg/day)} × 6.25 (g protein/day)" and normalized by body weight (g protein/kg body weight/day).

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), and podocalyxin was measured with human ELISA kit (Cat No. CSB-E09891h; Cusabio Biotech) according to the manufacturers' protocols. All cytokine concentrations were measured in duplicate and adjusted for urinary creatinine concentration.

Statistical analysis

The urine marker-to-creatinine ratio (a unit/g creatinine) was multiplied by calculating the estimated creatinine excretion rate (eCER) (g/24 hour) to obtain the estimated urine excretion of a marker (in a unit/24 hour)[16]. The equations suggested by Tanaka et al [17] were used to calculate eCER. Protein intake was calculated as follows: "24-hour urine urea nitrogen [17] + {body weight [18] × 0.031 (g nitrogen/kg/day)} × 6.25 (g protein/day)" and normalized to body weight (g protein/kg body weight/day). Differences in the parameters between 16- and 0-week values were calculated, as well as the percentage change; "(value at 16-week − value at 0-week) × 100/value at 0-week (%)". Decreases in albuminuria and urine cytokines were defined based on the ratios of estimated albuminuria and urine cytokines at the 16-week time point compared with those at the 0-week time point and were ≤ −25%.

All analyses and calculations were performed using SPSS Statistics ver. 21.0 (IBM Co., Armonk, NY, USA). Continuous variables were expressed as the mean ± standard deviation or as a percentage for categorical variables. The Kruskal–Wallis test was used to compare continuous variables between 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 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 mean age at enrollment was 50.3 ± 13.0 years, and 50% of participants were male. The mean BMI was 25.4 ± 3.8 kg/m^2, and approximately 53.7% of subjects were obese (BMI ≥ 25 kg/m^2) (Supplementary Table 1; available at https://doi.org/10.23876/j.krcp.2018.37.1.49). Participants were assigned to three groups according to the change in body weight after 16 weeks. Fifty-eight participants (25.6%) were assigned to group 1 (≥ 1.5%

Table 2. Percent change in parameters over 16 weeks according to change in body weight

Parameter	Group 1 (n = 58)	Group 2 (n = 32)	Group 3 (n = 136)	P value
Difference in body weight* (kg)	−2.41 ± 1.45	−0.55 ± 0.33	1.28 ± 1.74	< 0.001
% change of 24-hr Una/Cr[†]	−15.2 ± 37.5	−5.3 ± 40.2	6.1 ± 52.7	0.017
% change of protein intake[†]	9.8 ± 58.5	18.9 ± 67.4	20.0 ± 75.5	0.988
% change of SBP[†]	−6.1 ± 12.5	−10.2 ± 7.9	−6.6 ± 10.9	0.232
% change of total cholesterol[†]	−7.9 ± 14.9	−3.4 ± 14.2	−1.7 ± 19.2	0.052
% change of eGFR[†]	−6.1 ± 14.6	−3.7 ± 13.3	−4.8 ± 13.6	0.633
Education group				
Intensive group (%)	67.2	50.0	40.4	0.003

Data are presented as mean ± standard deviation or percent only.
Group 1, patients with decreased body weight ≥ 1.5% during 16 weeks; group 2, patients with decreased body weight of 1.5—0.1% during 16 weeks; group 3, patients with increased body weight ≥ 0.0% during 16 weeks.
Cr, creatinine; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; Una, urine sodium.
P values for continuous variables were estimated by Kruskal–Wallis test, and P values for categorical variables were calculated by Pearson's chi-square test.
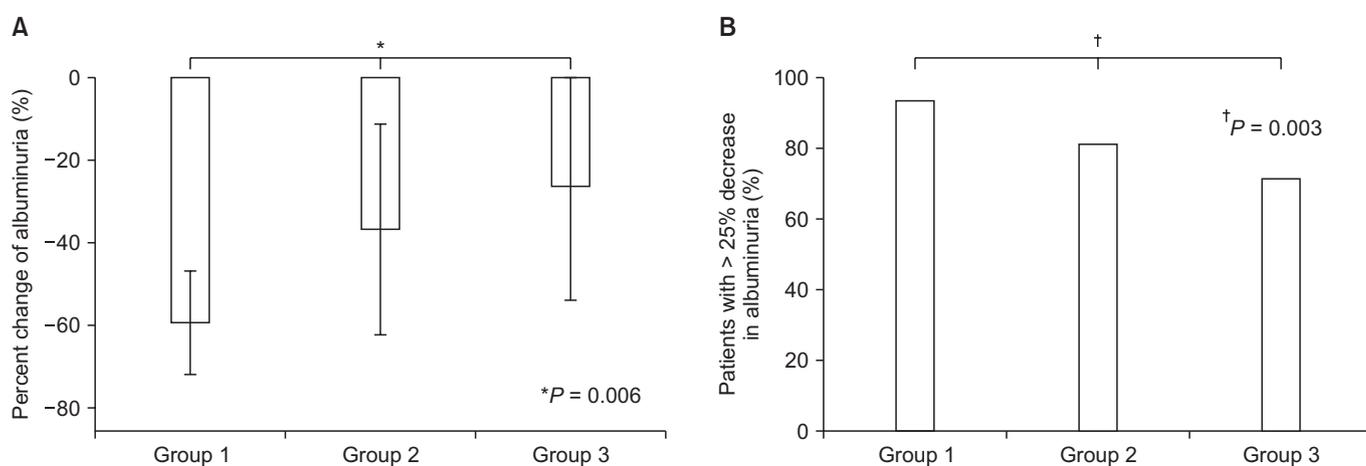*Body weight at 16 weeks — body weight at 0 weeks.
[†]The ratio of 24-hr Una/Cr (estimated 24-hr urine sodium to creatinine ratio, mEq/g Cr), protein intake (g/kg/day), SBP (mmHg), total cholesterol (mg/dL), eGFR (mL/min/1.73 m^2) were calculated, as well as the percentage change; (value at 16-week — value at 0-week) × 100/value at 0-week (%).

decrease in body weight after 16 weeks), 32 participants (14.1%) were assigned to group 2 (1.5−0.1% decrease in body weight after 16 weeks), and 136 participants (59.9%) were assigned to group 3 (≥ 0.0% increase in body weight after 16 weeks) (Table 1). Group 3 had the highest mean age, total cholesterol level, eGFR, and amount of estimated protein intake using the estimated level of urine urea nitrogen, as well as the lowest mean BMI at enrollment. However, the differences were not statistically significant. Other baseline characteristics at enrollment including BP, comorbidities, and medication use were also not different among the three groups.

The mean differences in weight over the period were statistically significant; −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 ($P <$ 0.001). The percent changes in other parameters during the 16 weeks were also evaluated according to group (Table 2). The ratios of estimated protein intake, systolic BP (SBP), total cholesterol, and eGFR between the 16- and 0-week time points did not significantly differ among the three groups. In other words, there was no evidence that clinical status was getting worse in the weight-loss group. However, the percent change in urinary sodium excretion, which indicates the change in salt intake over the 16 weeks, was significantly different among the three groups ($P = 0.017$), and the proportion of change among the intensive education group was remarkably higher for those who lost weight, as expected ($P = 0.003$).

Changes in body weight and decreases in albuminuria

Next, the percent change in albuminuria over the 16 weeks was compared among the three groups using the Kruskal−Wallis test (Fig. 2A). With a greater decrease in body weight over the 16 weeks, there was a greater decrease in albuminuria. The percentage of participants who achieved > 25% decrease in 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 (Fig. 2B). Multiple logistic regression analysis was also used to evaluate the independent factors for predicting > 25% decrease in albuminuria after 16 weeks adjusting for age, sex, and other factors related to decreased albuminuria level, such as albuminuria at baseline; percent changes in SBP, diastolic BP, eGFR, total CO_2, serum total cholesterol, and 24-hour urine excretion of sodium; and group change in body weight (Table 3). As shown in Table 3, the probability of decreased albuminuria with ARB treatment was higher in the study population with baseline albuminuria and reduced body weight. In particular, the participants in group 1 (with ≥ 1.5% decrease in body weight after 16 weeks) showed the highest probability of decreased albuminuria level. The interactions were explored between change 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

Figure 2. Decreased albuminuria according to change in body weight during the 16-week study period. (A) Percent change in albuminuria over 16 weeks. (B) Percentage of participants who achieved > 25% decrease in albuminuria. Group 1 (≥ 1.5% decrease in body weight), group 2 (1.5−0.1% decrease in body weight), and group 3 (≥ 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 and †Pearson's chi-square test.

Table 3. Independent factors to predict > 25% decrease in albuminuria over 16 weeks

Variable	B	Wald	RR	95% CI of RR	P value
Albuminuria at baseline* (mg/d)	0.212	4.781	1.236	1.022–1.494	0.029
Percent change of SBP (%)	−0.062	13.237	0.940	0.910–0.972	< 0.001
Percent change of 24-hr Una†(%)	−0.009	6.235	0.991	0.984–0.998	0.013
Group of change in body weight‡		9.217			0.010
Group 1	1.830	9.217	6.234	1.913–20.315	0.002
Group 2	0.188	0.133	1.207	0.438–3.326	0.716

B, logistic coefficients; CI, confidence interval; RR, relative risk; SBP, systolic blood pressure; Una, urine sodium; Wald, test statistics for individual predictor value.

Group 1, patients with decreased body weight ≥ 1.5% during 16 weeks; group 2, patients with decreased body weight of 1.5—0.1% during 16 weeks.

P values were estimated by multiple logistic regression analysis adjusted for age, gender, season at enrollment, and related factors to achieve a decrease in albuminuria, such as albuminuria at baseline; percent changes in SBP, DBP, eGFR, total CO_2, serum total cholesterol, and 24-hr urine excretion of sodium; and change in body weight group.

*Estimated 24-hr albumin excretion, obtained using estimated daily creatinine excretion from the Tanaka equation.

†Estimated 24-hr sodium excretion obtained using estimated daily creatinine excretion from the Tanaka equation.

‡Group of change in body weight compares the relative risk of achieving a decrease in albuminuria to group 3.
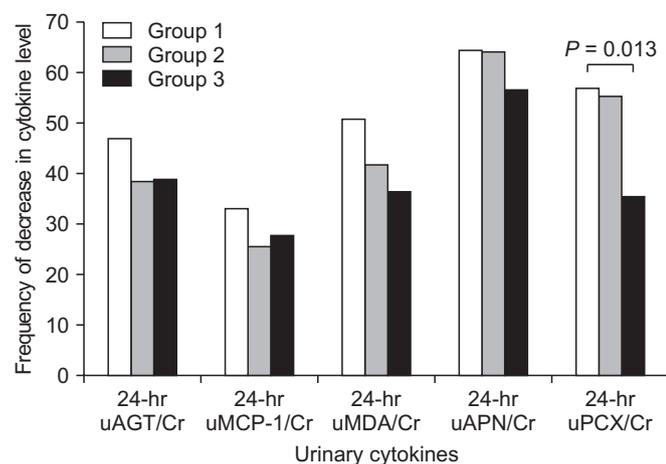
Figure 3. Changes in urinary cytokines according to change in body weight over 16 weeks. Frequency of decrease in cytokine level: The frequency of a 25% or greater reduction in 24-hour urinary cytokine to creatinine ratio at 16 weeks compared to 0 weeks. Group 1 (≥ 1.5% decrease in body weight), group 2 (1.5—0.1% decrease in body weight), and group 3 (≥ 0.0% increase in body weight).

AGT, angiotensinogen; APN, adiponectin; Cr, creatinine; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; PCX, podocalyxin; u-, urine.

between changes in body weight and the other independent factors.

Changes in body weight and decreases in urinary cytokines

Finally, correlations between the frequencies of reduced cytokines and changes in weight were assessed.

Participants with reduced body weight had a higher frequency of decreases in the following urinary cytokines after 16 weeks: adiponectin, AGT, MCP-1, MDA, and podocalyxin (Fig. 3). However, only the decrease in podocalyxin, a marker of podocyte injury, was statistically significant across groups ($P = 0.013$). Next, factors related to the decrease in podocalyxin were examined. Among baseline clinical parameters, estimated urine albumin excretion was an independent risk factor. The ratio of estimated urine albumin excretion at the 16-week time point compared to the 0-week time point also showed the probability of a decrease in podocalyxin (Supplementary Table 2; available at https://doi.org/10.23876/j.krcp.2018.37.1.49).

Discussion

In this subanalysis, we demonstrate that even minimal unintentional weight reduction had additive effects on the anti-proteinuric effects of ARBs in hypertensive CKD patients. Over the study period, weight loss independently increased the probability of reduced albuminuria. Among the urinary cytokines evaluated to elucidate mechanisms, only podocalyxin was significantly decreased in participants who lost weight. Furthermore, both estimated urine albumin excretion at 0 weeks and the ratio of urine albumin excretion at 16 weeks compared to 0 weeks were independent risk factors for decreased urinary podocalyxin.

As mentioned above, many studies have explored the

relationship between weight loss and CKD progression. Overweight and obese weight categories 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 [10,19—21]. Recently, obesity itself has been proposed as an independent risk factor for CKD and ESRD in several observational studies [10,22,23]. Lifestyle changes such as calorie- and salt controlled diets and regular exercise, which can lead to weight reduction, have been considered important management strategies for CKD [5,12]. In the clinical trial on which this study was based, participants were intensively or conventionally educated about low-salt diets. Some patients unintentionally lost weight during the study period. Even if the degree of weight loss was minimal, weight reduction showed an additive effect on the anti-proteinuric effects of ARBs in hypertensive CKD patients. There was no evidence that weight reduction during the study period was related to disease progression.

Being obese or overweight leads to alterations in renal hemodynamics characterized by increased glomerular filtration pressure and perfusion [24—26]. Obesity also augments sympathetic activity [27], activates the RAS [28,29], activates insulin resistance [30], increases inflammation and oxidative stress [31], and attenuates the bioactivity of nitric oxide [32,33]. Furthermore, levels of adipose tissue-derived adipokines, which influence podocyte biology, are altered in obese patients [34]. Adiponectin, an adipokine, is decreased in obese patients, and its levels can be normalized by weight reduction [35]. Based on this pathophysiology, the beneficial effects of weight reduction on renal damage induced by being overweight might be due to decreases in hyperfiltration, filtration pressure, inflammation, and oxidative stress, as well as increases in nitric oxide bioactivity and adiponectin level. Therefore, weight loss could effectively decrease proteinuria.

In this study, we measured urinary biomarkers such as AGT as an index of intrarenal RAS status, MDA as a marker of oxidative stress, MCP-1 as an index of inflammation status, adiponectin, and podocalyxin as a marker of podocyte injury. Among these urinary biomarkers, only podocalyxin significantly decreased with the percent change in body weight over 16 weeks (Fig. 3). Albuminuria also significantly decreased with the percent change in body weight (Fig. 2). Through this study, we observed a close relationship between unintentional weight loss and improved albuminuria and also between albuminuria and urinary podocalyxin excretion. Consequently, we suggest that weight loss reduces albuminuria as a result of improved glomerular injury by ameliorating podocyte damage.

Reduction in proteinuria is one of the most valuable treatments for CKD patients, and it is achieved by pharmacological and non-pharmacological management methods [36—38]. The results of this study, which showed an additive beneficial effect of unintentional weight reduction on treatment of albuminuria in non-obese and obese hypertensive CKD patients, are very meaningful. Weight loss had a significant additive effect on reduction in proteinuria, even if weight reduction during the study period was minimal. Low salt and protein restricted diets, as well as weight loss, are related to decreased proteinuria. However, we demonstrated that minimal weight reduction independently reduced proteinuria. In addition, the decreased proteinuria induced by weight loss was related to decreased podocyte damage.

This study also has some limitations. First, we used 24-hour urine specimens and measured body weight 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. Weight measurement was also influenced by the time of measurements, weight scale, and apparel. Because this was a multicenter outpatient-based study, weight scales were different for each institution, and the time at which weight was measured differed according to clinic visit. However, weight loss was associated with decreased proteinuria regardless of seasonal changes. Second, this study was not designed to explore the effects of dietary changes 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. In particular, we could not distinguish between a loss of volume and solid weight. Third, only one parameter was used for each signal of RAS, inflammation, reactive oxygen species, and podocyte injury, which reduces the accuracy for detecting signal in the kidney. Although 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 cytokines were

measured 6 months after the first urine collection period. During this period, urine contents could have been altered in each sample, introducing an accuracy error.

In conclusion, this study demonstrated that weight loss had an additive effect on the anti-proteinuric effects of treatment with ARBs in nondiabetic hypertensive CKD patients, even though the degree of weight loss was minimal. Those effects were demonstrated in both obese and non-obese participants. Possible mechanisms are related to reduced podocyte damage. Based on these results, a well-designed clinical trial is needed to elucidate the effect of weight change on the prognosis of CKD patients.

Conflicts of interest

All authors have no conflicts of interest to declare.

Acknowledgments

References

[1] Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 351:1296-1305, 2004

[2] Tonelli M, Wiebe N, Culleton B, et al. Chronic kidney disease and mortality risk: a systematic review. *J Am Soc Nephrol* 17:2034-2047, 2006

[3] Polonsky KS. The past 200 years in diabetes. *N Engl J Med* 367:1332-1340, 2012

[4] Danaei G, Finucane MM, Lin JK, et al. National, regional, and global trends in systolic blood pressure since 1980: systematic analysis of health examination surveys and epidemiological studies with 786 country-years and 5•4 million participants. *Lancet* 377:568-577, 2011

[5] James MT, Hemmelgarn BR, Tonelli M. Early recognition and prevention of chronic kidney disease. *Lancet* 375:1296-1309, 2010

[6] Saran R, Li Y, Robinson B, et al. US renal data system 2014 annual data report: epidemiology of kidney disease in the United States. *Am J Kidney Dis* 66(1 Suppl 1):Svii, S1-S305, 2015

[7] Aitken GR, Roderick PJ, Fraser S, et al. Change in prevalence of chronic kidney disease in England over time: comparison of nationally representative cross-sectional surveys from 2003 to 2010. *BMJ Open* 4:e005480, 2014

[8] Wouters OJ, O'Donoghue DJ, Ritchie J, Kanavos PG, Narva AS. Early chronic kidney disease: diagnosis, management and models of care. *Nat Rev Nephrol* 11:491-502, 2015

[9] Jain N, Reilly RF. Effects of dietary interventions on incidence and progression of CKD. *Nat Rev Nephrol* 10:712-724, 2014

[10] Wang Y, Chen X, Song Y, Caballero B, Cheskin LJ. Association between obesity and kidney disease: a systematic review and meta-analysis. *Kidney Int* 73:19-33, 2008

[11] Hsu CY, McCulloch CE, Iribarren C, Darbinian J, Go AS. Body mass index and risk for end-stage renal disease. *Ann Intern Med* 144:21-28, 2006

[12] Menzin J, Lines LM, Weiner DE, et al. A review of the costs and cost effectiveness of interventions in chronic kidney disease: implications for policy. *Pharmacoeconomics* 29:839-861, 2011

[13] Hwang JH, Chin HJ, Kim S, et al. Effects of intensive low-salt diet education on albuminuria among nondiabetic patients with hypertension treated with olmesartan: a single-blinded randomized, controlled trial. *Clin J Am Soc Nephrol* 9:2059-2069, 2014

[14] Casas JP, Chua W, Loukogeorgakis S, et al. Effect of inhibitors of the renin-angiotensin system and other antihypertensive drugs on renal outcomes: systematic review and meta-analysis. *Lancet* 366:2026-2033, 2005

[15] Kawasaki T, Uezono K, Itoh K, Ueno M. [Prediction of 24-hour urinary creatinine excretion from age, body weight and height of an individual and its application]. *Nihon Koshu Eisei Zasshi* 38:567-574, 1991. In Japanese

[16] Fotheringham J, Campbell MJ, Fogarty DG, El Nahas M, Ellam T. Estimated albumin excretion rate versus urine albumin-creatinine ratio for the estimation of measured albumin excretion rate: derivation and validation of an estimated albumin excretion rate equation. *Am J Kidney Dis* 63:405-414, 2014

[17] Tanaka T, Okamura T, Miura K, et al. A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. *J Hum Hypertens* 16:97-103, 2002

[18] Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P; Acute Dialysis Quality Initiative Workgroup. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second

International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 8:R204-R212, 2004

[19] Adams KF, Schatzkin A, Harris TB, et al. Overweight, obesity, and mortality in a large prospective cohort of persons 50 to 71 years old. *N Engl J Med* 355:763-778, 2006

[20] Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature* 444:875-880, 2006

[21] Stengel B, Tarver-Carr ME, Powe NR, Eberhardt MS, Brancati FL. Lifestyle factors, obesity and the risk of chronic kidney disease. *Epidemiology* 14:479-487, 2003

[22] Wahba IM, Mak RH. Obesity and obesity-initiated metabolic syndrome: mechanistic links to chronic kidney disease. *Clin J Am Soc Nephrol* 2:550-562, 2007

[23] Hall JE, Crook ED, Jones DW, Wofford MR, Dubbert PM. Mechanisms of obesity-associated cardiovascular and renal disease. *Am J Med Sci* 324:127-137, 2002

[24] Ribstein J, du Cailar G, Mimran A. Combined renal effects of overweight and hypertension. *Hypertension* 26:610-615, 1995

[25] Porter LE, Hollenberg NK. Obesity, salt intake, and renal perfusion in healthy humans. *Hypertension* 32:144-148, 1998

[26] Chagnac A, Weinstein T, Korzets A, Ramadan E, Hirsch J, Gafter U. Glomerular hemodynamics in severe obesity. *Am J Physiol Renal Physiol* 278:F817-F822, 2000

[27] Landsberg L, Krieger DR. Obesity, metabolism, and the sympathetic nervous system. *Am J Hypertens* 2:125S-132S, 1989

[28] Ahmed SB, Fisher ND, Stevanovic R, Hollenberg NK. Body mass index and angiotensin-dependent control of the renal circulation in healthy humans. *Hypertension* 46:1316-1320, 2005

[29] Hopkins PN, Lifton RP, Hollenberg NK, et al. Blunted renal vascular response to angiotensin II is associated with a common variant of the angiotensinogen gene and obesity. *J Hypertens* 14:199-207, 1996

[30] Rocchini AP. Obesity hypertension, salt sensitivity and insulin resistance. *Nutr Metab Cardiovasc Dis* 10:287-294, 2000

[31] Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. *Gastroenterology* 132:2169-2180, 2007

[32] Sansbury BE, Hill BG. Regulation of obesity and insulin resistance by nitric oxide. *Free Radic Biol Med* 73:383-399, 2014

[33] Colomba D, Duro G, Corrao S, et al. Endothelial nitric oxide synthase gene polymorphisms and cardiovascular damage in hypertensive subjects: an Italian case-control study. *Immun Ageing* 5:4, 2008

[34] Wickman C, Kramer H. Obesity and kidney disease: potential mechanisms. *Semin Nephrol* 33:14-22, 2013

[35] Serra A, Granada ML, Romero R, et al. The effect of bariatric surgery on adipocytokines, renal parameters and other cardiovascular risk factors in severe and very severe obesity: 1-year follow-up. *Clin Nutr* 25:400-408, 2006

[36] de Zeeuw D. Should albuminuria be a therapeutic target in patients with hypertension and diabetes? *Am J Hypertens* 17:11S-15S; quiz A2-4, 2004

[37] Jafar TH, Stark PC, Schmid CH, et al. Progression of chronic kidney disease: the role of blood pressure control, proteinuria, and angiotensin-converting enzyme inhibition: a patient-level meta-analysis. *Ann Intern Med* 139:244-252, 2003

[38] Stenvinkel P. Chronic kidney disease: a public health priority and harbinger of premature cardiovascular disease. *J Intern Med* 268:456-467, 2010