Anti-albuminuric effect of the aldosterone blocker eplerenone in non-diabetic hypertensive patients with albuminuria: a double-blind, randomised, placebo-controlled trial

*Katsuyuki Ando, Hiroshi Ohtsu, Shunya Uchida, Shinya Kaname, Yoshihiro Arakawa, Toshiro Fujita, for the EVALUATE Study Group**

Lancet Diabetes Endocrinol 2014; 2: 944–53

Published Online October 28, 2014 http://dx.doi.org/10.1016/S2213-8587(14)70194-9

See Comment page 925

*Members listed in appendix

The University of Tokyo, Tokyo, Japan (K Ando MD, Y Arakawa PhD, T Fujita MD); **Juntendo University, Tokyo, Japan** (H Ohtsu MS); **Teikyo University, Tokyo, Japan** (S Uchida MD); **and Kyorin University, Tokyo, Japan** (S Kaname MD)

Correspondence to: Dr Toshiro Fujita, Division of Clinical Epigenetics, Research Center for Advanced Science and Technology (RCAST), The University of Tokyo, Tokyo, Japan **toshiro.fujita@rcast.u-tokyo.ac.jp**

See Online for appendix

Summary

Background Renin-angiotensin system inhibitors have renoprotective effects in patients with chronic kidney disease, but most patients treated with these drugs have residual urinary albumin excretion. Some small clinical studies show that mineralocorticoid receptor blockade reduces albuminuria. Our study aimed to examine the beneficial effects of addition of a selective aldosterone antagonist, eplerenone, to renin-angiotensin system inhibitors in hypertensive patients with non-diabetic chronic kidney disease.

Methods In this double-blind, randomised, placebo-controlled trial, we enrolled hypertensive patients, aged 20–79 years, with albuminuria (urinary albumin-to-creatinine ratio [UACR] in the first morning void urine of 30–599 mg/g), an estimated glomerular filtration rate of 50 mL/min per 1·73 m² or more, and who had received an angiotensin-converting enzyme inhibitor, an angiotensin receptor blocker, or both, for at least 8 weeks. Participants were from 59 clinics and hospitals in Japan. Eligible patients were randomly assigned (1:1), stratified by baseline characteristics, to either low-dose eplerenone (50 mg/day) or placebo, with continuation of standard antihypertensive treatment to attain therapeutic goals (<130/80 mm Hg) for 52 weeks. We assessed efficacy in all patients who received allocated treatment, provided a baseline and post-treatment urine sample, and remained in follow-up. We assessed safety in all patients who received allocated treatment. The primary efficacy measure was percent change in UACR in the first morning void urine at week 52 from baseline. The trial is registered at the clinical trials registry of University Hospital Medical Information Network (UMIN), trial identification number UMIN000001803.

Findings Between April 1, 2009, and March 31, 2012, we randomly allocated 170 patients to the eplerenone group and 166 patients to the placebo group. In the primary efficacy analysis, mean percent change in UACR from baseline was –17·3% (95% CI –33·65 to –0·94) for 158 patients in the eplerenone group compared with 10·3% (–6·75 to 22·3) for 146 patients in the placebo group (absolute difference –27·6% [–51·15 to –3·96]; p=0·0222). In the safety analyses, 53 (31%) of 169 patients in the eplerenone group had adverse events (five serious), as did 49 (30%) of 163 in the placebo group (seven serious). Although mean serum potassium concentration was higher in the eplerenone group than the placebo group, severe hyperkalaemia (>5·5 mmol/L) was not recorded in either group.

Interpretation Addition of low-dose eplerenone to renin-angiotensin system inhibitors might have renoprotective effects through reduction of albuminuria in hypertensive patients with non-diabetic chronic kidney disease, without serious safety concerns.

Funding Pfizer.

Introduction

Hypertension and albuminuria are known risk factors for cardiovascular and renal events. The renin-angiotensin system (RAS) inhibitors, including angiotensin receptor blockers and angiotensin-converting enzyme inhibitors, have renoprotective and blood pressure-lowering effects; thus, guidelines recommend RAS inhibitors as first-line antihypertensive agents for patients with chronic kidney disease. In patients with non-diabetic nephropathy, inhibition of RAS activity reduces urinary protein excretion and slows the decline of the glomerular filtration rate.[1] Renoprotective effects might be at least partly independent of blood pressure-lowering effects, as the effects of RAS inhibitors are greater than those of other antihypertensive drugs at similar levels of blood pressure control.[2,3] However, hypertensive patients with chronic kidney disease treated with a RAS inhibitor often have substantial residual urinary albumin excretion. Because albuminuria is a crucial predictor of poor renal outcomes,[2,4,5] more extensive treatment in these patients is necessary.

Accumulating evidence, especially from animal studies, has shown that aldosterone plays a crucial part in renal injury and that blockade of the mineralocorticoid receptor, an aldosterone receptor, is renoprotective.[6,7] Activation of the mineralocorticoid receptor in the kidney may be independent of plasma aldosterone concentrations, as shown in a rat model of chronic kidney disease where a mineralocorticoid receptor antagonist suppressed the progression of renal injury in both high[6] and low[7] plasma aldosterone levels. Thus, a mineralocorticoid receptor

antagonist might have beneficial effects on renal injury in patients with chronic kidney disease who are already treated with RAS inhibitors. Indeed, several small-scale clinical studies have suggested a renoprotective effect of such antagonists. The selective mineralocorticoid receptor antagonist eplerenone was superior to a calcium antagonist in elderly patients with hypertension and micro-albuminuria,[8] and to an angiotensin-converting enzyme inhibitor in hypertensive patients with albuminuria.[9] In addition, renal protection is seen with the addition of a mineralocorticoid receptor antagonist to an angiotensin-converting enzyme inhibitor in patients with diabetic nephropathy.[10–13] However, high doses of eplerenone (up to 200 mg), or of a more potent but less selective mineralocorticoid receptor antagonist spironolactone, were used in most studies to date, and resulted in an increased risk of severe hyperkalaemia in patients treated with RAS inhibitors. Notably, reduced doses of eplerenone (25–50 mg/day), that do not induce severe hyperkalaemia, suppressed urinary albumin excretion in elderly patients treated with angiotensin receptor blockers or angiotensin-converting enzyme inhibitors.[14] However, this study enrolled few patients and treatment with mineralocorticoid receptor antagonists was only for a short period.

Our study aimed to investigate the effects of long-term, low-dose eplerenone (50 mg/day) added to standard therapy with an angiotensin-converting enzyme inhibitor, angiotensin receptor blocker, or both, on the urinary albumin-to-creatinine ratio (UACR) in hypertensive patients with non-diabetic chronic kidney disease.

Methods

Study design and participants

The Eplerenone Combination versus Conventional Agents to Lower Blood Pressure on Urinary Anti-albuminuric Treatment Effect (EVALUATE) study was a prospective, multicentre, double-blind, placebo-controlled, randomised trial that assessed the effects of the mineralocorticoid receptor antagonist eplerenone on albuminuria in hypertensive patients with non-diabetic chronic kidney disease.[15] The study was investigator-driven and managed by the EVALUATE steering committee.

Patients from 59 clinics and hospitals in Japan participated and were enrolled in this study. Eligible patients were aged 20–79 years, were hypertensive with systolic blood pressures of 130–179 mm Hg or diastolic blood pressures of 80–99 mm Hg, had pre-treatment UACR in the first morning void urine (a mean of three measurements in three consecutive visits) of 30–599 mg/g, had an estimated glomerular filtration rate (eGFR) of 50 mL/min per 1·73 m² or more, and had received an angiotensin-converting enzyme inhibitor, an angiotensin-receptor blocker, or both, for at least 8 weeks. Major exclusion criteria included hypertensive emergencies that required intravenous administration of antihypertensive agents; serum potassium concentrations of 5·0 mmol/L or more; diabetes (fasting blood glucose concentration ≥126 mg/dL or treatment with anti-diabetic drugs); severe liver damage (Child-Pugh score: class C); severe heart failure (New York Heart Association class ≥III); severe arrhythmia (frequent ventricular or atrial extrasystole, prolonged ventricular tachycardia, atrial tachyarrhythmia with severe tachycardia, atrial fibrillation or flutter with severe tachycardia, sick sinus syndrome with severe bradycardia, or atrioventricular block with severe bradycardia); angina; myocardial infarction or cerebrovascular disease within 6 months before registration; pregnancy, possibility of pregnancy, or a desire to become pregnant; a history of severe adverse effects from mineralocorticoid receptor antagonists, angiotensin-converting enzyme inhibitors, or angiotensin-receptor blockers; administration of a mineralocorticoid receptor antagonist less than 8 weeks before registration; taking contraindicated drugs (including adrenocorticosteroidal drugs, immunosuppressants, potassium-sparing diuretics, potassium supplementation, itraconazole, ritonavir, and nelfinavir); and treatment for more than 2 weeks with non-steroidal anti-inflammatory drugs at registration. The study protocol was approved by the institutional review board of the University of Tokyo Clinical Research Center (reference number P2008028-11X) as the central review board and by review boards of other participating hospitals. The study was done in full accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained after the participants received verbal and written explanations about the study from the attending physicians. We followed the Consolidated Statement of Reporting Trials (CONSORT) guidelines.[16]

Randomisation and masking

After confirmation of eligibility, we randomly allocated patients in a 1:1 ratio to either the eplerenone (50 mg/day) group or placebo group with a centralised computer-generated allocation procedure stratified by the following patient characteristics: UACR (<300 mg/g *vs* ≥300 mg/g), systolic blood pressure (<140 mm Hg *vs* ≥140 mm Hg), and previous treatment with the RAS inhibitors (angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, *vs* dual treatment with angiotensin-converting enzyme inhibitor plus angiotensin receptor blocker). We adopted the web-based allocation system used by UMIN, a data centre run as a public institution in Japan. The allocation table, which was created by stratified block randomisation (block size 4) by the trial statistician, was registered with UMIN. Block size was concealed to all investigators until code breaking.

DBcaps capsules (Capsugel Japan, Sagamihara, Japan) were used to mask the test drugs (eplerenone and placebo). Encapsulated study drugs were prepared and packaged centrally by the pharmacy of the University of Tokyo and distributed to the participating hospitals. The study investigators, patients, data collection and management personnel, and statisticians were all masked to the treatment assignment throughout the study.

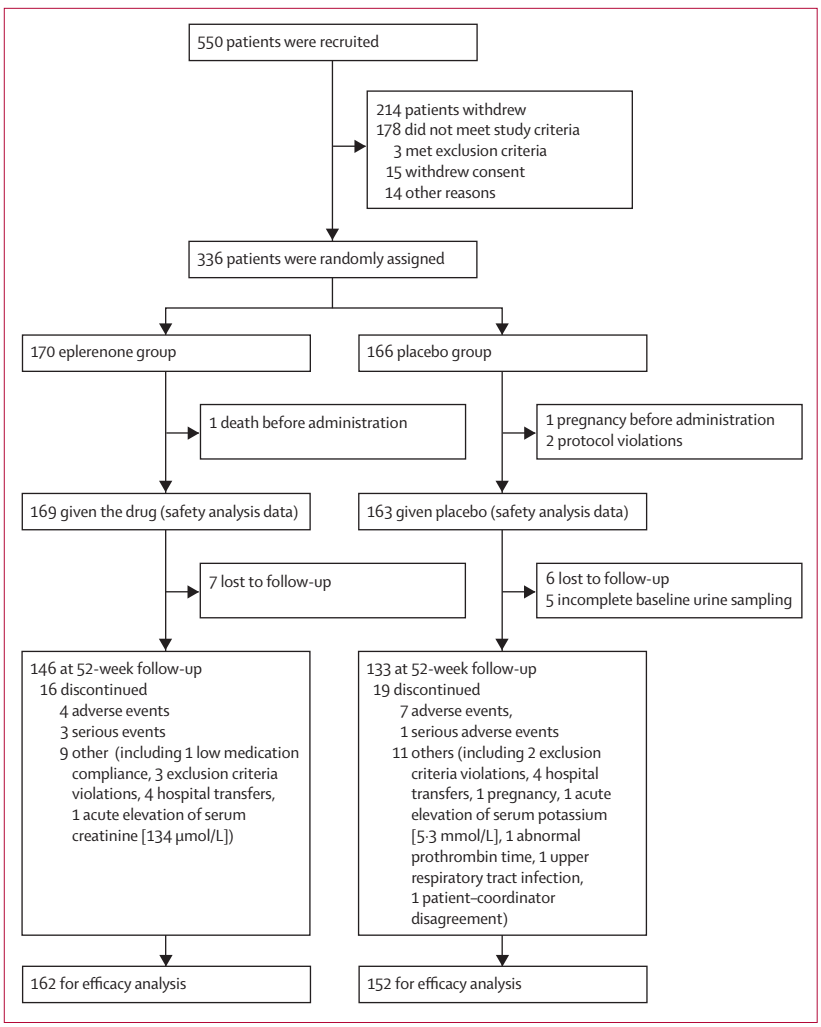

Figure 1: **Trial profile**

Procedures

Patients received assigned treatments for 52 weeks, and antihypertensive treatments received at baseline were continued. Other usual care medications were allowed throughout the study. If blood pressure was 130/80 mm Hg or more, the addition of antihypertensive medication (apart from a mineralocorticoid receptor antagonist, angiotensin-converting enzyme inhibitor, or angiotensin receptor blocker) was allowed to reach the treatment goal. Changes to RAS inhibitor drug classes and their doses was not allowed after the stratified randomisation.

Patient follow-up visits were at 4, 8, 16, 28, 40, and 52 weeks after initiation of the study drug. Office standard cuff blood pressure, blood samples, and urine specimens (first morning void urine), and adverse effects were assessed at every visit. Urinary albumin, liver-type fatty acid-binding protein (L-FABP), sodium, and creatinine were measured at the central laboratory (SRL, Tokyo, Japan), and the other measurements measured at each clinic. Data were collected via the University Medical Information Network Internet Data and Information Center for Medical Research (UMIN INDICE) system (Tokyo, Japan).

Outcomes

The primary efficacy measure was percent change from baseline in UACR in the first morning void urine at 52 weeks or last visit in patients who discontinued. Secondary endpoints were absolute values and percent changes from baseline at 4, 8, 28, and 52 weeks in the UACR in first morning void urine, serum creatinine concentrations, eGFR, urinary L-FABP, estimated 24-h urinary sodium excretion, and the incidence of cerebrovascular and cardiovascular events. Changes in plasma and urinary aldosterone were prespecified secondary outcomes, but on-treatment values were variable and meaningless, so we report only baseline values. We also assessed the safety profile of eplerenone treatment with the endpoints of changes in serum potassium concentrations and incidence of adverse effects. We calculated eGFR with the modification in diet in renal disease formula modified by the Japanese Society of Nephrology.[17] We estimated 24-h urinary sodium excretion with a previously reported formula.[18] Cerebrovascular and cardiovascular events included deaths (fatal myocardial infarction, fatal heart failure, sudden death, fatal stroke, and other cardiovascular deaths) and hospital admission (non-fatal myocardial infarction, angina, heart failure, cerebral bleeding, cerebral infarction, and transient ischaemic attack) from these causes.

Statistical analysis

We calculated the sample size on the assumption that the treatment for hypertensive patients with albuminuria who were receiving RAS inhibitors decreases urinary albumin concentration (primary efficacy measure) by 45%, whereas RAS inhibitor plus eplerenone would decrease it by 74%.[19] A sample size of 340 patients (170 patients per group) provided 80% power to detect a 30% reduction in urinary albumin concentration in the eplerenone group compared with that in the placebo group, with a significance level of 0·05 and accounting for a dropout rate of 10%.

Efficacy analyses included all randomly allocated patients who received at least one dose of study drug who had a valid baseline and at least one valid post-baseline assessment. For safety assessments, data from all patients who took the study drug at least once were included in the analyses. We compared differences in the primary efficacy measure (percent change in UACR after 52 weeks [or last visit] from baseline) between the eplerenone and placebo groups with one-way ANOVA. We analysed the secondary outcome measures in the same way. eGFR, 24-h urinary sodium excretion, and serum potassium concentration were analysed at each visit. Additionally, we assessed both the percent changes

in UACR and changes in blood pressure from baseline with the area under the curve technique. In post-hoc analyses, we compared percent change in UACR between groups by dividing patients into two groups according to baseline urinary sodium excretion (≥160 mmol/day and <160 mmol/day). We also assessed the correlation of percent change in UACR with baseline urinary sodium excretion, baseline plasma and urinary aldosterone, percent decrease in systolic blood pressure, and percent decrease in eGFR, with hypothesis tests of correlation. All analyses were two-sided and the significance level was 0·05. Statistical analyses were done by a trial statistician (HO) with the JMP Pro software version 10.0.2 (SAS Institute).

This trial was registered with the clinical trials registry of University Hospital Medical Information Network (UMIN), number UMIN000001803.

	Eplerenone group (n=162)	Placebo group (n=152)
Sex		
Male	114 (70%)	100 (66%)
Female	48 (30%)	52 (34%)
Age (years)	58·6 (13·0)	58·6 (13·8)
Height (cm)	163·8 (9·4)	162·2 (9·3)
Bodyweight (kg)	68·7 (14·5)	68·0 (13·0)
Cause of chronic kidney disease		
Hypertensive nephrosclerosis	91 (56%)	99 (65%)
Primary glomerular disease	63 (39%)	45 (30%)
Other	8 (5%)	8 (5%)
Office blood pressure (mm Hg)		
Systolic	138·6 (11·1)	138·8 (12·6)
Diastolic	82·4 (10·2)	81·9 (10·0)
Pulse rate (beats per min)	72·4 (10·1)	72·1 (11·1)
Laboratory data		
UACR (mg/g)	163·1 (148·0)	156·8 (133·6)
BUN (mg/dL)	15·3 (4·5)	15·5 (4·0)
Serum creatinine (μmol/L)	77·3 (17·2)	74·8 (14·9)
eGFR (mL/min per 1·73 m^2)*	67·7 (14·3)	68·6 (13·6)
Blood sugar (mg/dL)	100·1 (11·1)	102·2 (10·9)
Triglycerides (mg/dL)	169·1 (137·8)	155·8 (96·5)
LDL cholesterol (mmol/L)	2·89 (0·709)	2·82 (0·714)
HDL cholesterol (mmol/L)	1·44 (0·422)	1·43 (0·422)
Serum sodium (mmol/L)	141·1 (2·2)	140·8 (2·1)
Serum potassium (mmol/L)	4·15 (0·36)	4·15 (0·40)
Estimated 24 h urinary sodium (mmol/day)	219·5 (66·1)	217·6 (58·1)
Urinary L-FABP/creatinine ratio (μg/g)	0·07 (0·37)	0·04 (0·12)
Plasma renin activity (ng/mL per h)	3·94 (4·97)	4·07 (4·93)
Plasma aldosterone (pg/mL)	89·0 (44·9)	90·1 (51·0)
Urinary aldosterone/creatinine ratio (ng/g)	0·038 (0·029)	0·044 (0·043)
(Continued from previous column)		
Cardiovascular diseases		
Myocardial infarction	0	0
Heart failure or arrhythmia	7 (4%)	8 (5%)
Stroke	0	0
Other atherosclerotic diseases	2 (1%)	2 (1%)
Concomitant treatments at baseline		
Antihypertensive drugs		
ARB†	148 (91%)	136 (89%)
ACE-I	20 (12%)	18 (12%)
Calcium-channel blocker	100 (62%)	95 (62%)
β-blocker or α-blocker and β-blocker	20 (12%)	22 (14%)
Diuretic		
Thiazide	23 (14%)	18 (11%)
Loop	2 (1%)	1 (<1%)
α-blocker	9 (6%)	7 (5%)
Lipid-lowering drugs		
Statins	52 (32%)	48 (32%)
Fibrates	8 (5%)	2 (1%)
Aspirin	10 (6%)	10 (7%)
Others	2 (1%)	1 (<1%)

Data are n (%) or mean (SD), unless otherwise stated. UACR=urinary albumin-to-creatinine ratio. BUN=blood urea nitrogen. eGFR=estimated glomerular filtration rate. L-FABP=liver-type fatty acid-binding protein. ARB=angiotensin receptor blocker. ACE-I=angiotensin-converting enzyme inhibitor. *eGFR was calculated with the modification of diet in renal disease formula modified by the Japanese Society of Nephrology.[16] †Six patients in the eplerenone group and two in the placebo group had ARB and ACE-I combination therapy.

Table 1: **Baseline characteristics of patients included in efficacy analyses**

Role of the funding source

EVALUATE was funded by Pfizer. Pfizer had no role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript. The principal investigator (TF) had full access to all data in the study and takes responsibility for the integrity of the data, the accuracy of the data analysis, and the final decision to submit for publication.

Results

Between April 1, 2009, and March 31, 2012, we enrolled 336 hypertensive patients with chronic kidney disease and randomly assigned 170 to the eplerenone group and 166 to the placebo group (figure 1, table 1). Of allocated patients, four were excluded before receipt of any study drug and thus 332 patients were included in the safety assessment (figure 1). During the study period, 13 patients were lost to follow-up, and five did not provide complete baseline urine sampling. Thus, we included 314 patients in efficacy assessments (figure 1). UACR was not measured at 52 weeks in ten of the 314 patients, so the primary endpoint (%UACR) was

	Eplerenone group			Placebo group			Maximum dose‡ (mg/day)
	n	Dose* (mg/day)	Dose category (n)†	n	Dose* (mg/day)	Dose category (n)†	
Angiotensin receptor blocker	148	..	41 high, 75 medium, 32 low	136	..	21 high, 76 medium, 39 low	..
Valsartan	41	90·24 (45·85)	11 high, 22 medium, 8 low	35	80 (38·81)	6 high, 18 medium, 11 low	160
Candesartan	32	8·75 (2·77)	11 high, 16 medium, 5 low	33	7·39 (2·42)	3 high, 23 medium, 7 low	12
Olmesartan	29	23·62 (12·02)	9 high, 13 medium, 7 low	31	22·58 (11·25)	8 high, 15 medium, 8 low	40
Telmisartan	24	40 (19·34)	4 high, 14 medium, 6 low	15	36 (21·31)	2 high, 7 medium, 6 low	80
Losartan	18	53·78 (28·30)	4 high, 8 medium, 6 low	16	45·31 (19·3)	1 high, 10 medium, 5 low	100
Irbesartan	4	150 (57·74)	2 high, 2 medium, 0 low	6	100 (54·77)	1 high, 3 medium, 2 low	200
Angiotensin-converting enzyme inhibitor	20	..	12 high, 2 medium, 6 low	18	..	4 high, 9 medium, 5 low	..
Enalapril	6	7·71 (3·74)	4 high, 1 medium, 1 low	4	5 (3·54)	1 high, 1 medium, 2 low	10
Imidapril	6	7·5 (3·87)	4 high, 0 medium, 2 low	5	3·25 (1·68)	0 high, 2 medium, 3 low	10
Perindopril	2	4, 4	0 high, 2 medium, 0 low	3	3·33 (1·15)	2 high, 1 medium, 0 low	8
Trandolapril	2	2, 2	2 high, 0 medium, 0 low	0	..	0 high, 0 medium, 0 low	2
Temocapril	1	2	0 high, 1 medium, 0 low	4	2·5 (1)	1 high, 3 medium, 0 low	4
Cilazapril	1	0·25	0 high, 0 medium, 1 low	0	..	0 high, 0 medium, 0 low	2
Lisinopril	1	2	0 high, 0 medium, 1 low	1	10	0 high, 1 medium, 0 low	20
Captopril	1	56·25	0 high, 0 medium, 1 low	0	..	0 high, 0 medium, 0 low	150
Benazepril	0	..	0 high, 0 medium, 0 low	1	5	0 high, 1 medium, 0 low	10

*Dose is mean (SD), but the dose for individual patients is shown where there are only one or two patients. †Dose categories were divided as high, medium, and low: medium dose is the standard dose shown in the drug package insert in Japan, high dose is higher than standard, and low dose is lower than standard. ‡Maximum dose approved in Japan.

Table 2: **Doses of angiotensin receptor blocker and angiotensin-converting enzyme inhibitor in patients included in the efficacy analyses**

assessed in 304 patients. For the same reason, 24-h urinary sodium excretion and urinary L-FABP were assessed in 307 and 289 patients, respectively.

Baseline demographic, clinical, and biochemical characteristics were well balanced between groups (table 1). Mean urinary sodium excretion was high in both groups (218 mmol/day); higher than mean daily sodium excretion (about 160 mmol/day) recorded in 129 global datasets.[20] Concomitant drug use at baseline and doses administered were also much the same (table 1). In terms of RAS inhibitors, about one quarter of patients in both groups were taking high doses, about one half were taking medium doses, and about one quarter were taking low doses (table 2).

The percent reduction in early morning UACR from baseline was significantly greater in the eplerenone group than in the placebo group after 52 weeks (between-group difference –27·6%; 95% CI –51·15 to 3·96; p=0·0222; table 3). The anti-albuminuric effect of eplerenone was observed early (from week 4) and continued until the end of treatment; percent change in UACR from baseline as assessed by area under the curve was significantly (p<0·0001) lower in the eplerenone group than the placebo group (figure 2).

In post-hoc analyses stratified by urinary sodium excretion, in patients with high urinary sodium excretion (≥160 mmol/day), percent change in UACR from baseline was significantly greater in those in the eplerenone group than in those in the placebo group, but we noted no significant difference in percent change in UACR between patients in the eplerenone group and those in the placebo group with low urinary sodium excretion (<160 mmol/day) (table 3). In the eplerenone group, there was no significant correlation of percent decrease in UACR with baseline urinary excretion (r=0·0400, p=0·619) or baseline concentrations of plasma (r=0·134, p=0·096) or urinary (r=–0·144, p=0·148) aldosterone. No change in urinary sodium excretion or urinary L-FABP in either group was recorded (table 3).

A small but significant reduction in eGFR was recorded at week 8 in the eplerenone group only, but eGFR did not further decrease in the remaining study period (table 3). Percent decrease in eGFR from baseline at 52 weeks was also greater in the eplerenone group than the placebo group (table 3). The proportion of patients at each chronic kidney disease stage, based on eGFR, before and after treatment, did not differ between the groups (table 4).

We noted differences in systolic blood pressure between the groups throughout the study (figure 3). Systolic blood pressure decreased from baseline by week 4 in the eplerenone group. Systolic blood pressure also decreased in the placebo group, but to a lesser

	Eplerenone group			Placebo group			Between-group difference		p value
	N	Mean	95% CI	N	Mean	95% CI	Mean	95% CI	
Primary endpoint*									
UACR change from baseline (%)	158	−17·3	−33·65 to −0·94	146	10·3	−6·75 to 22·3	−27·6	−51·15 to −3·96	0·0222
Secondary endpoint*									
eGFR change from baseline (%)	156	−4·6	−7·07 to −2·19	151	0·47	−2·00 to 2·96	−5·1	−8·58 to −1·63	0·0041
24-h UNa change from baseline (mmol/day)	159	0·12	−12·21 to 12·44	148	6·4	−6·37 to 19·18	−6·3	−24·05 to 11·46	0·486
Urinary L-FABP change from baseline (μg/L)	150	−0·29	−1·67 to 1·08	139	0·62	−0·814 to 2·045	−0·91	−2·896 to 1·073	0·367
Urinary L-FABP/creatinine change from baseline (%)	150	−2·01	−19·55 to 15·53	139	14·03	−4·19 to 32·26	−16·04	−41·34 to 9·253	0·213
Safety analysis†									
Serum potassium change from baseline (mmol/L)	158	0·17	0·102 to 0·244	151	0·02	−0·048 to 0·097	0·14	0·057 to 0·250	0·0043
Comparison of groups at each visit									
eGFR (mL/min per 1·73 m²)									
8 weeks	148	64·8	62·27 to 67·33	135	69·0	66·38 to 71·67	−4·2	−7·88 to −0·56	0·0241
28 weeks	147	64·6	62·18 to 66·96	133	68·3	65·77 to 70·80	−3·7	−7·19 to −0·25	0·0358
52 weeks	146	64·1	61·56 to 66·67	138	68·0	65·39 to 70·64	−3·9	−7·56 to −0·23	0·0372
Serum potassium (mmol/L)									
4 weeks	154	4·30	4·24 to 4·37	147	4·15	4·089 to 4·213	0·15	0·240 to 0·067	0·0006
8 weeks	148	4·31	4·25 to 4·37	135	4·20	4·139 to 4·266	0·11	0·022 to 0·198	0·0142
28 weeks	146	4·34	4·28 to 4·40	133	4·19	4·125 to 4·259	0·15	0·054 to 0·240	0·0020
52 weeks	146	4·32	4·25 to 4·340	133	4·16	4·082 to 4·233	0·17	0·062 to 0·270	0·0019
24-h UNa (mmol/day)									
8 weeks	148	209·6	195·90 to 217·31	136	218·0	206·85 to 229·19	−11·4	−26·89 to 4·01	0·148
28 weeks	147	207·2	196·50 to 217·95	133	212·0	200·70 to 223·25	−4·7	−30·31 to 10·81	0·549
52 weeks	146	223·0	211·71 to 234·34	133	218·2	206·33 to 230·04	4·8	−11·55 to 21·22	0·562
Post-hoc subgroup analysis*									
UACR change from baseline (%)									
24-h UNa <160 mmol/day	31	−6·0	−30·82 to 18·79	23	6·7	−22·13 to 35·46	−12·7	−50·68 to 25·33	0·506
24-h UNa ≥160 mmol/day	127	−20·0	−39·41 to −0·69	123	10·9	−8·74 to 30·61	−31·0	−58·58 to −3·38	0·0280

UACR=urinary albumin-to-creatinine ratio. eGFR=estimated glomerular filtration rate. UNa=urinary sodium excretion. L-FABP=liver-type fatty acid-binding protein. *At 52 weeks, or last visit for efficacy assessment in patients who discontinued the study. †At 52 weeks, or last visit for safety assessment.

***Table 3*: Changes in variables from baseline values in patients included in efficacy and safety analyses**

extent than in the eplerenone group; similar changes were recorded for diastolic blood pressure. Percent reduction in UACR correlated with percent decrease in systolic blood pressure (r=0·140, p=0·0153), suggesting that the anti-albuminuric effects of eplerenone might be dependent on blood pressure reduction. However, percent reduction in UACR did not correlate with percent decrease in eGFR (r=0·0007, p=0·990).

In safety analyses, seven (4%) of 169 patients in the eplerenone group and eight (5%) of 163 patients in the placebo group discontinued the study drugs because of adverse events (figure 1, table 5). Incidence of serious and non-serious adverse events did not differ between groups (table 5). There was one death in the eplerenone group from unknown causes, cardiovascular and cerebrovascular events were rare, with no difference between groups (table 5). Serum potassium concentrations throughout the study were slightly but significantly higher in the eplerenone group than the placebo group (table 3). Notably, no patient in either group had hyperkalaemia, defined as a potassium concentration greater than 5·5 mmol/L, although 15 patients who received eplerenone and four patients who received placebo had a serum potassium concentration of 5·1–5·5 mmol/L. Two of 15 patients in the eplerenone group had sustained increases in serum potassium concentration, whereas others had transient increases during the treatment period.

Discussion

In our trial, addition of low-dose eplerenone (50 mg/day), a selective aldosterone receptor antagonist, to RAS inhibitor treatment in patients with non-diabetic chronic kidney disease with albuminuria reduced UACR compared with placebo (panel). Notably, residual albuminuria is a strong predictor of adverse renal outcomes in long-term studies of patients with chronic kidney disease treated with RAS inhibitors,[2–5,21,22] and so, reduction of albuminuria might be of benefit. Moreover, early reduction of 24-h urinary protein excretion with a RAS inhibitor reportedly correlated with the long-term effect on creatinine concentrations or end-stage renal

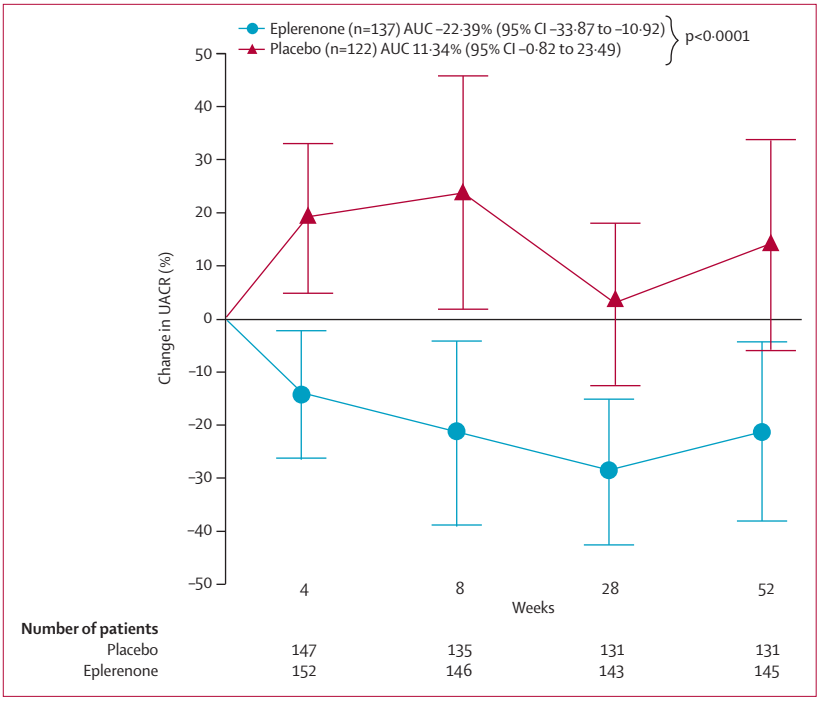

Figure 2: **Time course of percent change from baseline in urinary albumin-to-creatinine ratio (UACR) during the study period**

Comparisons between eplerenone and placebo groups: p=0·0005 (4 weeks), p=0·0015 (8 weeks), p=0·0022 (28 weeks), p=0·0079 (52 weeks). Data are means and 95% CIs. Time course during the study quantified as the area under the curve (AUC) of serial assessments from baseline to week 52.

	Baseline		Study completion	
	Eplerenone group (n=159)	Placebo group (n=151)	Eplerenone group (n=158)	Placebo group (n=152)
1	12 (8%)	13 (9%)	8 (5%)	16 (11%)
2	86 (54%)	88 (58%)	77 (49%)	82 (54%)
3a	59 (37%)	45 (30%)	61 (39%)	45 (30%)
3b	2 (1%)	5 (3%)	11 (7%)	9 (6%)
4	0	0	1 (1%)	0
Missing	3 (2%)	1 (<1%)	4 (3%)	0

Data are n (%). Stage 1: estimated glomerular filtration rate (eGFR) ≥90 mL/min per 1·73 m^2; stage 2: 60 mL/min per 1·73 m^2 ≤ eGFR < 90 mL/min per 1·73 m^2; stage 3a: 45 mL/min per 1·73 m^2 ≤ eGFR < 60 mL/min per 1·73 m^2; Stage 3b: 30 mL/min per 1·73 m^2 ≤ eGFR <45 mL/min per 1·73 m^2; stage 4: 15 mL/min per 1·73 m^2 ≤ eGFR < 30 mL/min per 1·73 m^2. CKD=chronic kidney disease.

Table 4: **Chronic kidney disease stages at baseline and study completion**

disease.[2] In our study, the anti-albuminuric effect of eplerenone was recorded in the early phase (4 weeks) of treatment and continued in the long term (52 weeks) without attenuation. In view of the hypothesis that albuminuria is sustained during long-term treatment of chronic kidney disease,[23] combination therapy with RAS blockade and a mineralocorticoid receptor blocker to further reduce albuminuria might prevent and slow progressive renal function loss.

With respect to the presence of residual albuminuria in patients with chronic kidney disease treated with RAS inhibitors, the occurrence of aldosterone breakthrough could be connected with resistance to RAS inhibitors. The incidence of aldosterone breakthrough ranges from 10% over 6 months to 53% over 1 year, as shown by a review of eight studies that enrolled patients with congestive heart failure in four, chronic kidney disease in three, and hypertension in one.[24] Treatment with spironolactone added to angiotensin-converting enzyme inhibitor apparently reduced UACR to a greater extent in 40% of patients with diabetes who developed breakthrough than the remaining patients without breakthrough.[11] Thus, addition of a mineralocorticoid receptor antagonist to RAS inhibitors is promising for the treatment of renal injury in patients with chronic kidney disease and aldosterone breakthrough. However, no reliable markers are available to predict the occurrence of breakthrough.

Regarding another possible cause of resistance to RAS inhibitors, urinary sodium excretion in patients recruited in this study (218 mmol/day) was higher than the mean of about 160 mmol/day recorded in 129 global datasets.[20] Moreover, anti-albuminuric effects of eplerenone were evident in patients with high concentrations of the estimated 24-h urinary sodium excretion (≥160 mmol/day) but not in those with low urinary sodium excretion (<160 mmol/day). Daily salt (NaCl) intake of greater than 11·7 g (equivalent to 200 mmol/g creatinine) seems to blunt the antiproteinuric effect of angiotensin-converting enzyme inhibitor therapy and increase the risk of end-stage renal disease, independent of blood pressure control.[25] Conversely, sodium depletion with a low-sodium diet or diuretic treatment is beneficial for individuals with proteinuria that are resistant to RAS blockade when previously on a high salt diet,[26] suggesting that knowledge of the salt intake of patients with chronic kidney disease is important in assessment of the potential antiproteinuric and renoprotective effects of RAS inhibitors. Regarding the putative mechanisms of salt-induced resistance to RAS inhibitors, high salt intake increased renal mineralocorticoid receptor activity despite decreased plasma aldosterone in salt-sensitive hypertensive rats,[27] possibly through an aldosterone-independent mineralocorticoid receptor-mediated pathway.[28] Because we noted no significant correlation of percent change in UACR with baseline plasma or urinary aldosterone in this study, it is possible that high salt-induced enhancement of mineralocorticoid receptor signalling at the level of the kidney is closely associated with the progression of renal injury in the setting of inadequate suppression of plasma aldosterone via aldosterone breakthrough, even if adequate suppression is provided by RAS inhibitors.

In our study, mean systolic blood pressure was lower in patients who received eplerenone than in those who received placebo after 4 weeks of eplerenone treatment,

an effect observed until study end at 52 weeks. Notably, the reduction of albuminuria was also recorded during the early period of eplerenone treatment, concomitant with blood pressure lowering. Consistent with changes in blood pressure, a significant reduction in eGFR also occurred early during eplerenone treatment, but this decline did not progress throughout the study, a result also shown by a previous study.[12] Thus, the early reduction of eGFR implies the functional effects of mineralocorticoid receptor antagonism. Mineralocorticoid receptor antagonists reduce the intraglomerular pressure via impaired tubuloglomerular feedback.[29] Functional reduction of eGFR induced by the decreased intraglomerular pressure might be renoprotective in the long term in most patients with chronic kidney disease, possibly through the inhibition of albuminuria.[23]

Serum potassium concentration was significantly increased during treatment with eplerenone, but no patients in either group had serum potassium concentrations greater than 5·5 mmol/L, suggesting that combination therapy with 50 mg/day eplerenone and standard doses of RAS inhibitors is safe for patients with chronic kidney disease, albuminuria, and eGFR of 50 mL/min per 1·73 m² or more. However, combination therapy with mineralocorticoid receptor antagonists and RAS inhibitors in patients with renal insufficiency increases the risk of serious, life-threatening hyperkalaemia. Thus, frequent measurement of serum potassium concentrations in all patients receiving combination treatment is mandatory.[30,31]

Our study has several limitations. The main limitation was the short treatment duration of 52 weeks. Despite 2·2 years of follow-up, a 2013 study did not show the renoprotective effects of dual therapy with an angiotensin receptor blocker and an angiotensin-converting enzyme inhibitor for the treatment of diabetic nephropathy despite the presence of antiproteinuric effects.[32] Notably, after 4 years of the STENO trial, in patients with diabetic nephropathy, intensive risk factor control did not decrease the risk of mortality despite reduced albuminuria, but after a 10-year follow-up, the intensive treatment reduced the risk of mortality and end-stage renal failure events.[33] Therefore, longer observation periods might be needed to assess the effects of study drugs on the prognosis of patients with renal dysfunction, and measurements of eGFR and the incidence of end-stage renal disease are necessary. In addition, background doses of angiotensin receptor blockers and angiotensin-converting enzyme inhibitors in our study were smaller compared with doses used in other trials that examined the effect of these drugs on urinary albumin excretion. Theoretically, maximum dose RAS inhibitors should be used whenever resistance to RAS inhibitors is assessed. However, full RAS system blockade may not be seen even with maximal doses of RAS inhibitors: the full antiproteinuric potential of the angiotensin receptor blocker irbesartan was not reached at the approved maximum dose of 300 mg once daily, and further increase of the dose up to 900 mg once daily resulted in a more complete RAS blockade and additional reduction in urinary albumin excretion.[34] Finally, we used

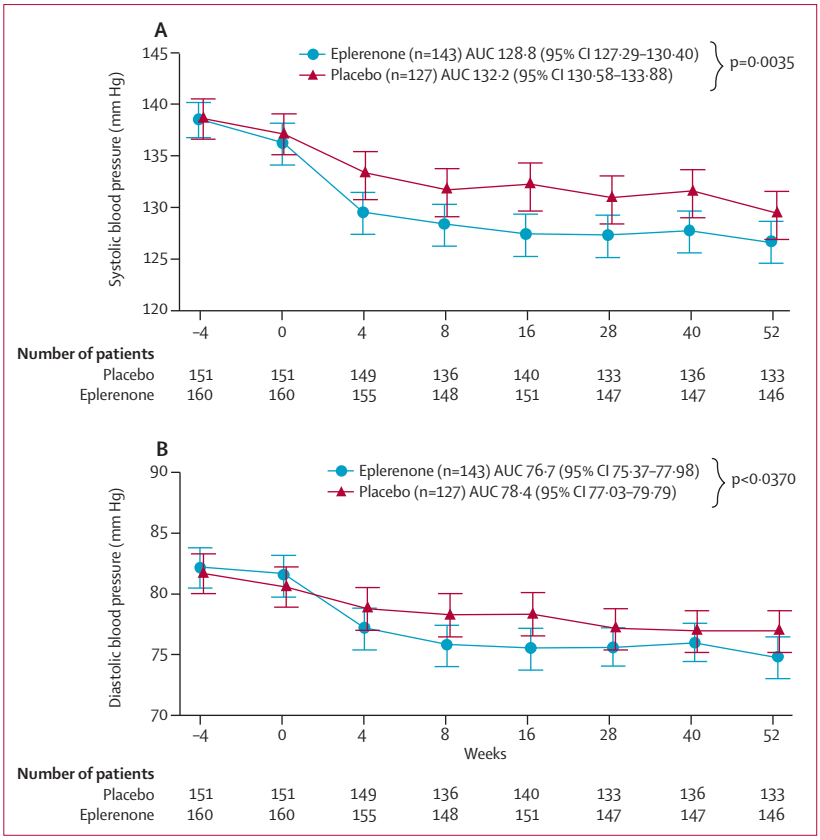

Figure 3: **Time course of office blood pressure during the study period**
Office (A) systolic and (B) diastolic blood pressures. Comparisons between eplerenone and placebo groups: (A) p=0·0149 (4 weeks), p=0·0289 (8 weeks), p=0·0018 (16 weeks), p=0·0227 (28 weeks), p=0·0137 (40 weeks), p=0·036 (52 weeks), and (B) p=0·0180 (16 weeks). Data are means and 95% CIs. Time course during the study quantified as the area under the curve (AUC) of serial assessments from baseline to week 52.

	Eplerenone group (n=169)	Placebo group (n=163)
Patients with adverse events	53 (31%)	49 (30%)
Serious adverse events	5 (3%)*	7 (4%)†
Non-serious adverse events	103 (61%)	104 (64%)
Dizziness	2 (1%)	6 (4%)
Tiredness	3 (2%)	3 (2%)
Digestive trouble	3 (2%)	1 (1%)
Headache	3 (2%)	1 (1%)
Increased liver enzymes	2 (1%)	1 (1%)
Hyperuricaemia	1 (1%)	1 (1%)
Others	89 (53%)	91 (56%)

Data are n (%). No patients were affected by hyperkalaemia, which was defined as a serum potassium level greater than 5·5 mmol/L. *Serious adverse events in the eplerenone group included sudden death (unknown causes), atrial fibrillation, renal dysfunction, gastric cancer, and liver dysfunction. †Serious adverse events in the placebo group included cerebrovascular infarction, colon cancer, bile duct cancer, uterine cervix cancer, colon polyp, bone fractures, and pyelonephritis.

Table 5: **Adverse events**

Panel: Research in context

Systematic review

We searched PubMed for randomised controlled trials published in English before January, 2009, and identified four articles that provided prospective data to assess the anti-albuminuric effects of mineralocorticoid receptor antagonists in patients with non-diabetic chronic kidney disease who received renin-angiotensin system (RAS) inhibitors. Addition of spironolactone to RAS inhibitors for the treatment of non-diabetic chronic kidney disease significantly reduced proteinuria by 37–70%. However, these studies enrolled few patients and none of the studies used the selective mineralocortcoid receptor antagonist, eplerenone. When preparing the report, our updated literature search identified an additional open-label trial, which showed that eplerenone, a mineralocorticoid receptor antagonist, reduced albuminuria in non-diabetic chronic kidney disease.

In patients with diabetic nephropathy, eight double-blind, placebo-controlled trials, most of which were small in size, showed that mineralocorticoid receptor antagonists reduced albuminuria in patients with diabetic nephropathy. We cited the most relevant articles. Systematic search of publications on "double-blind, placebo-controlled trial" with "eplerenone" or "spironolactone" in non-diabetic chronic kidney disease retrieved no articles.

Interpretation

In this double-blind, randomised, placebo-controlled trial, we compared eplerenone 50 mg with placebo in 314 hypertensive patients with albuminuria who were treated with standard doses of RAS inhibitors. Eplerenone reduced albuminuria to a greater extent than placebo in the early phase and the effect was continued to 52 weeks. Anti-albuminuric effects of eplerenone were recorded in patients with higher sodium excretion (≥160 mmol/day) but not in those with lower sodium excretion (<160 mmol/day), suggesting that high dietary sodium intake causes resistance to RAS inhibitors through mineralocorticoid receptor-mediated activation. Our results are based on surrogate endpoints; whether the observed effects will translate into improved clinical outcomes needs prospective testing in an appropriately sized outcomes study.

a formula to estimate sodium excretion in a single sample of morning fasting urine because this method is considered an adequate substitute for 24-h urinary sodium excretion.[35] Salt intake can be assessed by measuring urinary sodium excretion collected during 24 h, but this method is impractical in clinical trials.

We conclude that low-dose eplerenone might be safe and efficacious as add-on treatment to RAS inhibitors for hypertensive patients with non-diabetic chronic kidney disease.

Contributors

KA was responsible for the protocol, protocol review, study coordination, and management, including ethical and regulatory approvals, drug sourcing and management, data review, and drafting and review of the manuscript. HO did the protocol review, statistical analysis, and drafted and reviewed the manuscript. SU and SK reviewed the protocol, recruited patients, followed the protocol, and reviewed the manuscript. YA did the protocol review and masking. TF did the executive coordination, protocol and document drafting, protocol review, study coordination and management, data review, and drafted and reviewed the manuscript.

Declaration of interests

KA has received research grants from Daiichi-Sankyo, Kyowa Hakko Kirin, Boehringer Ingelheim, and Shionogi Pharm. HO has received honoraria from Statocom, EPS Corporation, Ministry of Health, Labour and Welfare, Daiichi-Sankyo, Chugai, Astellas, and Mitsubishi-Tanabe Pharm. SK has received research grants from Chugai, Genzyme Japan, Takeda, and Otsuka Pharm. YA has received a research grant from Toray. TF has received research grants and honoraria from Astellas, Toray, Boehringer Ingelheim, Chugai, Fukuda Denshi, Kyowa Hakko Kirin, Mitsubishi Tanabe, Mochida, Omron, Novartis, Pfizer, and Takeda Pharm. SU declares no competing interests.

Acknowledgments

A full list of the investigators and institutions is in the appendix. We thank Akira Yamada, Takuhiro Yamaguchi, Kiyoshi Kubota, and Tatsuo Shimosawa for their contribution to the success of this trial.

References

1 Ihle BU, Whitworth JA, Shahinfar S, Cnaan A, Kincaid-Smith PS, Becker GJ. Angiotensin-converting enzyme inhibition in nondiabetic progressive renal insufficiency: a controlled double-blind trial. *Am J Kidney Dis* 1996; **27:** 489–95.

2 The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia). Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. *Lancet* 1997; **349:** 1857–63.

3 Ruggenenti P, Perna A, Gherardi G, Gaspari F, Benini R, Remuzzi G, on behalf of Gruppo Italiano di Studi Epidemiologici in Nefrologia (GISEN). Renal function and requirement for dialysis in chronic nephropathy patients on long-term ramipril: REIN follow-up trial. *Lancet* 1998; **352:** 1252–56.

4 Lewis EJ, Hunsicker LG, Bain RP, Rohde RD, for the Collaborative Study Group. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. *N Engl J Med* 1993; **329:** 1456–62.

5 de Zeeuw D, Remuzzi G, Parving HH, et al. Proteinuria, a target for renoprotection in patients with type 2 diabetic nephropathy: lessons from RENAAL. *Kidney Int* 2004; **65:** 2309–20.

6 Nagase M, Yoshida S, Shibata S, et al. Enhanced aldosterone signalingin the early nephropathy of rats with metabolic syndrome: possible contribution of fat derived factors. *J Am Soc Nephrol* 2006; **17:** 3438–46.

7 Nagase M, Shibata S, Yoshida S, Nagase T, Gotoda T, Fujita T. Podocyte injury underlies the glomerulopathy of Dahl salt-hypertensive rats and is reversed by aldosterone blocker. *Hypertension* 2006; **47:** 1084–93.

8 White WB, Duprez D, St Hillaire R, et al. Effects of the selective aldosterone blocker eplerenone versus the calcium antagonist amlodipine in systolic hypertension. *Hypertension* 2003; **41:** 1021–26.

9 Williams GH, Burgess E, Kolloch RE, et al. Efficacy of eplerenone versus enalapril as monotherapy in systemic hypertension. *Am J Cardiol* 2004; **93:** 990–96.

10 Rossing K, Schjoedt KJ, Smidt UM, Boomsma F, Parving HH. Beneficial effects of adding spironolactone to recommended antihypertensive treatment in diabetic nephropathy: a randomized, double-masked, cross-over study. *Diabetes Care* 2005; **28:** 2106–12.

11 Sato A, Hayashi K, Naruse M, Saruta T. Effectiveness of aldosterone blockade in patients with diabetic nephropathy. *Hypertension* 2003; **41:** 64–68.

12 Epstein M, Williams GH, Weinberger M, et al. Selective aldosterone blockade with eplerenone reduces albuminuria in patients with type 2 diabetes. *Clin J Am Soc Nephrol* 2006; **1:** 940–51.

13 Mehdi UF, Adams-Huet B, Raskin P, Vega GL, Toto RD. Addition of angiotensin receptor blockade or mineralocorticoid antagonism to maximal angiotensin-converting enzyme inhibition in diabetic nephropathy. *J Am Soc Nephrol* 2009; **20:** 2641–50.

14 Yano Y, Hoshide S, Tamaki N, et al. Efficacy of eplerenone added to renin-angiotensin blockade in elderly hypertensive patients: the Jichi-Eplerenone Treatment (JET) study. *J Renin Angiotensin Aldosterone Syst* 2011; **12:** 340–47.

15 Ando K, Ohtsu H, Arakawa Y, et al. Rationale and design of the Eplerenone Combination Versus Conventional Agents to Lower Blood Pressure on Urinary Antialbuminuric Treatment Effect (EVALUATE) trial: a double-blinded randomized placebo-controlled trial to evaluate the antialbuminuric effects of an aldosterone blocker in hypertensive patients with albuminuria. *Hypertens Res* 2010; **33:** 616–21.

16 Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. *Ann Intern Med* 2010; **152:** 726–32.

17 Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009; **53:** 982–92.

18 Kawasaki T, Itoh K, Uezono K, Sasaki H. A simple method for estimating 24 h urinary sodium and potassium excretion from second morning voiding urine specimen in adults. *Clin Exp Pharmacol Physiol* 1993; **20:** 7–14.

19 Hollenberg NK. Aldosterone in the development and progression of renal injury. *Kidney Int* 2004; **66:** 1–9.

20 McCarron DA, Kazaks AG, Geerling JC, Stern JS, Graudal NA. Normal range of human dietary sodium intake: a perspective based on 24-hour urinary sodium excretion worldwide. *Am J Hypertens* 2013; **26:** 1218–23.

21 Jafar TH, Schmid CH, Landa M, et al. Angiotensin-converting enzyme inhibitors and progression of nondiabetic renal disease. A meta-analysis of patient-level data. *Ann Intern Med* 2001; **135:** 73–87.

22 Levey AS, Cattran D, Friedman A, et al. Proteinuria as a surrogate outcome in CKD: report of a scientific workshop sponsored by the National Kidney Foundation and the US Food and Drug Administration. *Am J Kidney Dis* 2009; **54:** 205–26.

23 de Zeeuw D, Parekh R, Soman S. CKD treatment: time to alter the focus to albuminuria? *Adv Chronic Kidney Dis* 2011; **18:** 222–23.

24 Bomback A, Klemmer PJ. The incidence and implications of aldosterone breakthrough. *Nat Clin Pract Nephrol* 2007; **3:** 486–92.

25 Vegter S, Perna A, Postma MJ, Navis G, Remuzzi G, Ruggenenti P. Sodium intake, ACE inhibition, and progression to ESRD. *J Am Soc Nephrol* 2012; **23:** 165–73.

26 Vogt L, Waanders F, Boomsma F, de Zeeuw D, Navis G. Effects of dietary sodium and hydrochlorothiazide on the antiproteinuric efficacy of losartan. *J Am Soc Nephrol* 2008; **19:** 999–1007.

27 Shibata S, Mu S, Kawarazaki H, et al. Rac1 GTPase in rodent kidneys is essential or salt-sensitive hypertension via a mineralocorticoid receptor-dependent pathway. *J Clin Invest* 2011; **121:** 3233–43.

28 Shibata S, Nagase M, Yoshida S, et al. Modification of mineralocorticoid receptor function by Rac1 GTPase: implication in proteinuric kidney disease. *Nat Med* 2008; **14:** 1370–76.

29 Fu Y, Hall JE, Lu D, et al. Aldosterone blunts tubuloglomerular feedback by activating macula densa mineralocorticoid receptors. *Hypertension* 2012; **59:** 599–606.

30 Epstein M. Hyperkalemia as a constraint to therapy with combination renin-angiotensin system blockade: the elephant in the room. *J Clin Hypertens* 2009; **11:** 55–60.

31 Shavit L, Lifschitz MD, Epstein M. Aldosterone blockade and the mineralocorticoid receptor in the management of chronic kidney disease: current concepts and emerging treatment paradigms. *Kidney Int* 2012; **81:** 955–68.

32 Fried LF, Emanuele N, Zhang JH, et al. Combined angiotensin inhibition for the treatment of diabetic nephropathy. *N Engl J Med* 2013; **369:** 1892–903.

33 Gaede P, Lund-Andersen H, Parving HH, Pedersen O. Effect of a multifactorial intervention on mortality in type 2 diabetes. *N Engl J Med* 2008; **358:** 580–91.

34 Rossing K, Schjoedt KJ, Jensen BR, Boomsma F, Parving HH. Enhanced renoprotective effects of ultra-high doses of irbesartan in patients with type 2 diabetes and microalbuminuria. *Kidney Int* 2005; **68:** 1190–98.

35 Mente A, O'Donnell MJ, Dagenais G, et al. Validation and comparison of three formulae to estimate sodium and potassium excretion from a single morning fasting urine compared to 24-h measures in 11 countries. *J Hypertens* 2014; **32:** 1005–15.