Beneficial impact of spironolactone on nephrotic range albuminuria in diabetic nephropathy

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Reduction of nephrotic range albuminuria is associated with markedly improved renal and cardiovascular outcome in patients with diabetic nephropathy. Aldosterone has been suggested to play a role in the progression of diabetic nephropathy. We therefore aimed to evaluate the short-term effect of aldosterone antagonism with spironolactone on nephrotic range albuminuria and blood pressure in diabetic nephropathy. Twenty Caucasian patients with diabetic nephropathy and nephrotic range albuminuria (>2500 mg/24 h) despite recommended antihypertensive treatment completed this double-masked, randomized crossover trial. Patients were treated in random order with spironolactone 25 mg once daily and matched placebo for 2 months, on top of ongoing antihypertensive treatment, including an angiotensin-converting enzyme inhibitor or an angiotensin II receptor blocker in maximally recommended doses. Median (range) number of antihypertensive drugs was 3 (2–5). After each treatment period, albuminuria, 24-h ambulatory blood pressure, and glomerular filtration rate (GFR) were determined. Spironolactone on top of recommended renoprotective treatment induced a 32% (95% confidence interval (CI): 21–42%) reduction in albuminuria from (geometric mean (95% CI)) 3718 (2910–4749) mg/24 h on placebo treatment ($P<0.001$). There was a significant reduction in 24-h blood pressure of 6 (2–10)/4 (2–6) mm Hg and day blood pressure of 7 (3–12)/5 (3–7) mm Hg ($P<0.01$), whereas night blood pressure remained unchanged. Spironolactone induced an insignificant reversible reduction in GFR of 3 ml/min/1.73 m^2 from 64 (27) ml/min/1.73 m^2. No patients were excluded due to adverse events. Our results suggest that spironolactone treatment on top of recommended renoprotective treatment including maximal renin–angiotensin system blockade may offer additional renoprotection in patients with diabetic nephropathy and nephrotic range albuminuria.

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Diabetic nephropathy has become the single most important cause of end-stage renal disease (ESRD) in the Western world.[1] Approximately one-third of all diabetic patients develop diabetic nephropathy. Before the introduction of aggressive antihypertensive treatment, the clinical course of diabetic nephropathy was characterized by a continuous increase in blood pressure and albuminuria, and an inevitable decline in glomerular filtration rate (GFR) of approximately 10–15 ml/min/year.[1] Thus, ESRD and death ensued on average within 5–7 years after the onset of diabetic nephropathy.[2] The fact that the degree of albuminuria is closely related to renal and cardiovascular outcome is well established,[3–6] and patients with nephrotic range proteinuria (proteinuria >3500 mg/24 h;[7] corresponding to albuminuria >2500 mg/24 h as reported previously[8]) indeed carry the highest risk of ESRD,[5] cardiovascular morbidity[6] and mortality, and consequently the shortest survival time.[9,10] Previously, the onset of nephrotic range albuminuria was considered to mark an inexorable progression to ESRD or death. It has however been demonstrated that remission of nephrotic range albuminuria is possible, and that it slows the progression in diabetic nephropathy, reduces the risk of ESRD, and improves survival in type I and type II diabetic patients with diabetic nephropathy.[9,10]

Recently, aldosterone antagonism has been shown to reduce albuminuria in diabetic and non-diabetic nephropathies.[11–14] However, the effect in patients with persistent nephrotic range albuminuria despite recommended renoprotective treatment has not been determined. In the present study, we evaluated the short-term effect of blocking aldosterone with spironolactone added to ongoing recommended renoprotective treatment including an angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB) and diuretics, on albuminuria and blood pressure in patients with diabetic nephropathy and nephrotic range albuminuria.

RESULTS

A total of 20 Caucasian patients were randomized in the trial; all of whom completed the study and were included in the statistical analysis. Baseline clinical data and baseline anti-hypertensive treatment are presented in Table 1.

Table 1 | Baseline clinical data and baseline antihypertensive treatment in 20 patients with diabetic nephropathy and nephrotic range albuminuria

	Type I diabetes (n=9)	Type II diabetes (n=11)
Age (years)	45 ± 8	52 ± 9
Gender (male/female)	8/1	9/2
Duration of diabetes (years)	30 ± 8	14 ± 8
Retinopathy, none/background/proliferative	0/4/5	2/6/3
Total number of antihypertensive drug, 2/3/4/5	5/1/3/0	1/5/4/1
Patients receiving		
RAS-blocking agent		
Enalapril 40/20 mg	3/3	3/0
Trandolapril 4 mg	—	1
Losartan 100/150 mg	2/0	3/1
Irbesartan 300 mg	—	2
Valsartan 160 mg	—	1
Dual blockade of RAS		
Lisinopril 20 mg+irbesartan 300 mg	1	—
Patients receiving diuretics, thiazide/loop diuretic	2/7	2/10[a]
Patients receiving dihydropyridines	3	9
Patients receiving β-blockers	2	6
Patients receiving moxonidine	1	0
Patients receiving statins	6	11
Patients receiving low-dose aspirin	6	10

RAS, renin–angiotensin system.
Data are expressed as *n*, mean ± s.d.
[a]One patient received both a thiazide and a loop diuretic.

The effects of spironolactone treatment are summarized in Table 2. During treatment with spironolactone in addition to conventional antihypertensive treatment, albuminuria was significantly reduced by 32% (95% confidence interval (CI): 21–42) compared to placebo treatment ($P < 0.001$; Table 2). The reduction in albuminuria was demonstrated in 17 of the 20 patients (Figure 1). There was a significant reduction in 24-h ambulatory blood pressure (ABP) of 6 (2–10)/4 (2–6) mm Hg and day ABP of 7 (3–12)/5 (3–7) mm Hg ($P < 0.01$; Table 2), whereas night ABP remained unchanged during spironolactone treatment.

There was a statistically insignificant trend toward a decline in GFR of 3 (−1 to 6) ml/min/1.73 m^2 and an increase in plasma creatinine of 8 (−4 to 20) μmol/l. The fractional clearance of albumin (θ_{alb}) was reduced by 31% (20–40) (Table 2).

There was a tendency toward an increase in plasma potassium during spironolactone treatment. Urinary sodium excretion, K/Na excretion rate, and plasma sodium were similar in the two treatment periods (Table 2). Plasma aldosterone levels increased by 80% (25–162) on spironolactone treatment compared to placebo treatment, and there was an increase in plasma renin activity (PRA) of 81% (25–162) during spironolactone treatment. There was a trend toward a decline in hemoglobin during treatment. HbA$_{1c}$ and

cholesterol levels remained unchanged during treatment (Table 2).

A subanalysis comparing type I and type II diabetic patients did not reveal any differences in response to spironolactone treatment in regard to changes in albuminuria, 24-h ABP, day ABP, or GFR ($P > 0.2$ for all comparisons, data not shown).

A total of five patients had albuminuria < 2.5 g/24 h during placebo treatment (Figure 1) despite nephrotic range albuminuria at randomization. When data from these five patients were excluded from the analysis, there was a reduction in albuminuria of 32% (95% CI 20–41%, $P < 0.001$), a reduction in 24-h ABP of 4 (0.3–8, $P = 0.04$)/3 (1–6, $P = 0.01$), and a reduction in day ABP of 6 (1–11, $P = 0.01$)/4 (2–7, $P = 0.002$). Night ABP remained unchanged and there was an insignificant reduction in GFR of 3 ml/min/1.73 m^2 ($P = 0.17$), that is, identical results in the remaining 15 patients as compared to $n = 20$.

Using linear regression analysis, we found no association between changes in albuminuria upon spironolactone treatment and baseline variables (placebo period). Baseline albuminuria was not significantly correlated to changes in albuminuria ($R = 0.21$, $P = 0.37$, that is, there was no order effect.

Changes in albuminuria were neither correlated to changes in systolic or diastolic 24-h ABP ($R < 0.34$, $P > 0.17$) nor to changes in systolic or diastolic day ABP ($R < 0.26$, $P > 0.3$) in univariate analyses. Furthermore, the reduction in albuminuria did not correlate significantly with changes in GFR.

There was no significant correlation between aldosterone levels at baseline and changes in albuminuria ($R = 0.33$, $P = 0.15$) or changes in blood pressure ($R < 0.3$, $P > 0.26$). Neither changes in plasma aldosterone nor in PRA correlated significantly to changes in albuminuria ($R = 0.22$, $P = 0.34$, and $R = 0.13$, $P = 0.59$, respectively). However, there was an inverse correlation between changes in PRA and changes in 24-h and day diastolic ABP ($R > 0.48$, $P < 0.05$), and a trend toward a similar inverse correlation between changes in plasma aldosterone levels and changes in day diastolic ABP ($R = 0.45$, $P = 0.06$), that is, the larger the compensatory increase in hormones, the larger the reduction in diastolic ABP.

The statistical analysis revealed no evidence of a time or carryover effect on albuminuria, ABP, GFR, PRA, or plasma aldosterone.

Throughout the study, all patients received renin–angiotensin system (RAS)-blocking treatment in recommended doses (Table 1); doses remained unchanged during the study. The median number of antihypertensive drugs in addition to the study medication was 3 (range 2–5). Compliance as assessed by tablet count was 99% (85–100), that is, all patients fulfilled the compliance criteria of $> 80\%$. Furthermore, compliance was assessed by evaluating the changes in plasma aldosterone and PRA. The three patients not responding to spironolactone treatment with a decline in albuminuria

Table 2 | Effects of adding spironolactone 25 mg o.d. to ongoing recommended antihypertensive treatment in 20 patients with diabetic nephropathy and nephrotic range albuminuria

	Conventional antihypertensive treatment		Mean difference (95% CI)	P-value
	+Placebo	+Spironolactone 25 mg		
Albuminuria (mg/24 h)[a]	3718 (2910–4749)	2510 (1831–3441)	−32% (−42 to −21)	<0.001
Systolic blood pressure (mm Hg)				
Office	146 (4)	142 (4)	−4 (−11 to 4)	0.32
24-h[b]	143 (3)	137 (3)	−6 (−10 to −2)	0.004
Day (7–23)[b]	147 (3)	140 (4)	−7 (−12 to −3)	0.002
Night (23–7)[c]	135 (3)	133 (4)	−2 (−7 to 2)	0.32
Diastolic blood pressure (mm Hg)				
Office	76 (2)	73 (2)	−3 (−6 to 0.4)	0.08
24-h[b]	81 (2)	77 (2)	−4 (−6 to −2)	0.001
Day (7–23)[b]	84 (2)	80 (2)	−5 (−7 to −3)	<0.001
Night (23–7)[c]	74 (2)	73 (2)	−1 (−4 to 2)	0.44
GFR (ml/min/1.73 m^2)	64 (2)	62 (2)	−3 (−6 to 1)	0.13
Fractional albumin clearance (θ_{alb}) ($\times 10^{-6}$)[a]	1.79 (1.25–2.56)	1.24 (0.82 to 1.87)	−31% (−40 to −20)	<0.001
Urinary sodium excretion (mmol/24 h)	239 (36)	210 (19)	−29 (−84 to 26)	0.28
Urinary K/Na ratio	0.43 (0.05)	0.43 (0.04)	0.01 (−0.06 to 0.07)	0.78
Bodyweight (kg)	93.8 (5.7)	92.6 (5.6)	1.1 (−0.1 to 2.4)	0.07
Plasma renin activity (ng AI/ml/h)[a]	8.9 (5.8–13.5)	16.1 (9.2–28.1)	81% (25–162)	0.003
Plasma aldosterone (pg/ml)[a]	38 (25–56)	68 (50–93)	80% (23–163)	0.004
Plasma creatinine (μmol/l)	141 (15)	149 (15)	8 (−4 to 20)	0.17
Plasma potassium (mmol/l)	4.1 (0.1)	4.3 (0.1)	0.2 (−0.004 to 0.5)	0.054
Plasma sodium (mmol/l)	139 (1)	138 (1)	−1 (−3 to 1)	0.28
Hemoglobin (mmol/l)	7.6 (0.2)	7.3 (0.2)	−0.2 (−0.5 to 0.01)	0.057
Hemoglobin A$_{1c}$ (%)	8.2 (0.3)	8.4 (0.3)	0.2 (−0.1 to 0.5)	0.20
Plasma cholesterol (mmol/l)	4.7 (0.3)	4.6 (0.2)	−0.1 (−0.4 to 0.3)	0.74
Plasma LDL cholesterol (mmol/l)	2.3 (0.2)	2.3 (0.2)	0.0 (−0.3 to 0.3)	0.88
Plasma HDL cholesterol (mmol/l)	1.5 (0.2)	1.5 (0.1)	−0.07 (−0.2 to 0.04)	0.20

GFR, glomerular filtration rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CI, confidence interval.
Data are expressed as mean (s.e.).
[a]Geometric mean (95% CI).
[b]$n=18$ because of insufficient measurements.
[c]$n=17$ because of insufficient measurements.

(Figure 1) all had an increase in plasma aldosterone of 30–147% and an increase in PRA of 110–200%.

Safety

No patients were excluded from the study. One patient complained of transient symptoms of orthostatic hypotension. Two patients were treated with kayexalate (sodium polystyrene sulfonate) due to slightly elevated plasma potassium (5.0–5.5). However, hyperkalemia was present in both treatment periods in one patient, and only in the placebo period in the other patient, that is, not specific for spironolactone treatment.

DISCUSSION

In our double-masked, randomized placebo-controlled crossover trial, we found that spironolactone on top of recommended renoprotective treatment reduced albuminuria and fractional albumin clearance in patients with diabetic nephropathy and nephrotic range albuminuria. Twenty-four-hour ABP was reduced by 6/4 mm Hg. Spironolactone treatment did not induce a significant reduction in GFR. The reduction in albuminuria was not significantly associated

with reduction in ABP. Furthermore, the albuminuria-lowering effect of aldosterone antagonism was independent of both baseline levels and changes in plasma aldosterone levels. However, the reduction in diastolic 24-h and day ABP was inversely associated with changes in PRA, that is, the larger the compensatory increase in PRA, the larger the reduction in diastolic ABP. Spironolactone treatment was generally well tolerated.

The present design of a double-masked, randomized, crossover study, lasting 2–3 months in each treatment arm has previously been applied successfully to evaluate new renoprotective drugs and combinations of drugs.[11,14–18]

It has recently been shown that aldosterone antagonism reduces albuminuria with or without concurrent blood pressure reduction in type I and type II diabetic patients with micro- or macroalbuminuria.[11–14] In the present study, we extended these observations to patients with diabetic nephropathy and nephrotic range albuminuria.

Previous studies in diabetic kidney disease have demonstrated that the maximal antiproteinuric effect occurs within 1–2 weeks after RAS blockade.[19,20] Similarly, the predominant antiproteinuric effect of RAS blockade in IRMA2[21] and
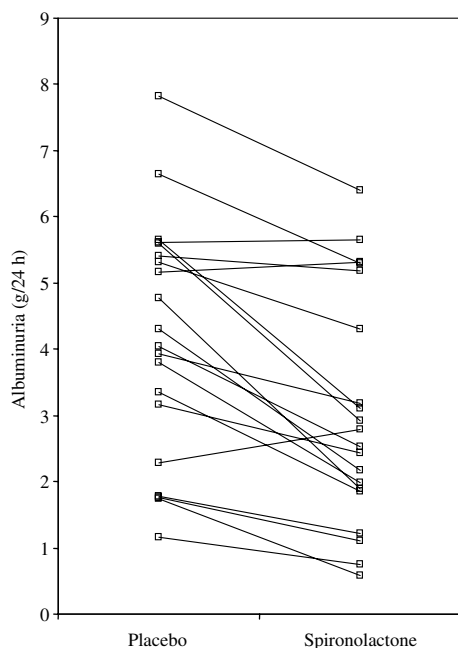
Figure 1 | Individual changes in albuminuria upon spironolactone treatment in 20 patients with diabetic nephropathy and nephrotic range albuminuria. Spironolactone was added to ongoing recommended renoprotective treatment.

RENAAL[22] was observed at the first measuring point (3–6 months). A small additional reduction in albuminuria occurred in both trials, simultaneously with an additional reduction in blood pressure. The effect on albuminuria of spironolactone in combination with ACEI or both ACEI and ARB has recently been reported to be maintained after 12 months treatment,[23] further suggesting a potential long-term beneficial effect. Several long-term studies have shown that the short-term reduction in albuminuria after initiation of antihypertensive treatment is a strong predictor of the long-term renal and cardiovascular outcome, that is, the patients with the greatest albuminuria reduction have the best long-term renal and cardiovascular outcome.[4–6,8–10,24] A reduction in albuminuria of 50% has been reported to be associated with a reduction in the risk of developing ESRD of 45% and heart failure of 27% in type II diabetic patients with diabetic nephropathy.[5,6] If we extrapolate from these data, the reduction in albuminuria of 32% observed in our study would translate into a relative risk reduction of 29% for progression to ESRD; however, long-term studies are needed in order to evaluate whether the short-term beneficial effect on the surrogate end point, albuminuria, is associated with the suggested reduction in development of ESRD and cardiovascular events.

There was no carryover effect on the albuminuria-lowering effect in our study, emphasizing that treatment effect is not maintained when treatment is interrupted, as previously shown for other RAS-blocking agents.[15,20]

All patients received RAS blockade with either ACEI or ARB. The doses used of the different drugs are regarded as the maximal recommended for blood pressure reduction.

Valid dose escalation studies using ultra-high doses of ACEIs and ARBs for renoprotection were not available when our study was conducted. Recent short-term studies of dual RAS blockade in type I and type II diabetic patients with nephropathy have demonstrated additional blood pressure and albuminuria-lowering effect.[17,18] Unfortunately, long-term studies dealing with safety and efficacy during treatment with ultra-high doses of ACEIs or ARBs or dual RAS blockade are lacking in diabetic nephropathy and guidelines do not recommend this type of therapy, which must be regarded as experimental. The recommended doses of ACEIs and ARBs have similar renoprotective effects.[16] All patients received diuretics in doses dependent on clinical effect and GFR level. Furthermore, additional antihypertensive agents (calcium channel blockers, β-blockers, or moxonidine) had been added individually, according to concomitant diseases, and tolerance to drug effects and side effects. On average, three blood pressure-lowering agents were prescribed. Consequently, our patients received optimal standard renoprotective treatment before entering the study. Furthermore, most patients received low-dose aspirin and statins (Table 1). It has previously been reported that a reduction in albuminuria is associated with a reduction in plasma cholesterol concentrations.[25,26] In our study, there were no changes in plasma cholesterol levels, probably due to rather low baseline cholesterol levels induced by treatment with statins.

In our study, there was a rather large interindividual variability in response to spironolactone treatment and three patients did not respond to treatment in terms of reduction in albuminuria, despite compliance to study medication as determined by marked increases in plasma aldosterone and PRA during treatment. It has been suggested that doubling of the spironolactone dose is effective in patients with essential hypertension, not responding to spironolactone 25 mg o.d.[27] This may also be the case in patients with diabetic nephropathy. However, one must be extremely cautious regarding hyperkalemia during treatment with spironolactone in patients with diabetic nephropathy and especially if higher doses are used. The effect of spironolactone on plasma potassium levels is dose dependent, as previously reported in the dose-finding study performed by the RALES investigators in 212 patients with chronic heart failure (NYHA classes II–IV) and reduced kidney function;[28] 80% of the patients who actually developed hyperkalemia (serum potassium $\geqslant 5.5$ mmol/l) did so within the first 8 weeks of spironolactone treatment. In the present study, we did not observe hyperkalemia caused by spironolactone. However, our patients were selected carefully, all having GFR > 30 ml/min/1.73 m^2 and plasma potassium < 4.5 mmol/l at baseline. Furthermore, all patients were on diuretic treatment (either loop or thiazide) and all patients received information about a low-potassium diet. Whether a clinically important accumulation of potassium occurs during long-term treatment with spironolactone in diabetic patients with nephrotic range albuminuria is unknown.

In the present study, effect of aldosterone antagonism did not depend on baseline levels of aldosterone, that is, even patients without aldosterone escape, as defined previously,[29] benefit from treatment. This is in agreement with findings in patients with essential hypertension treated with spironolactone.[27] Patients with and without primary aldosteronism responded similarly well on low-dose spironolactone (12.5–50 mg) with a mean reduction in blood pressure of 25/12 mm Hg after 6 months treatment, although patients with primary aldosteronism were more likely to have their dose of spironolactone uptitrated.[27]

Five of the participating patients had albuminuria < 2.5 g/ 24 h during placebo treatment despite nephrotic range albuminuria in the ⩾3 most recent 24-h urine collections at randomization. There can be a number of reasons for this. Three of the patients received spironolactone in the first treatment period, and an individual carryover effect implicating a prolonged effect of spironolactone cannot be excluded although there was no evidence for an overall carryover effect. Another explanation could be improved compliance to ongoing antihypertensive treatment during the study period, due to increased attention to ones own condition by the patient. Finally, regression toward the mean could explain the lower levels of albuminuria; this is however less likely, as we consistently measured albuminuria on three consecutive 24-h urine collections to minimize this problem.

In our study, proteinuric type I and type II diabetic patients were grouped together as previous biopsy studies have reported that structural changes are similar.[1] Furthermore, presence of diabetic retinopathy strongly suggests that a diabetic glomerulopathy is the cause of albuminuria.[30] All patients except two (where renal biopsy confirmed the diagnosis) had diabetic retinopathy. Finally, there appears to be no substantial difference between patients with type I and type II diabetes with respect to progression and treatment of diabetic nephropathy.[31] We recently published results from two studies, evaluating the renoprotective effect of spironolactone in type I[11] and type II[14] diabetic patients with diabetic nephropathy and lower grade albuminuria. These studies showed that type I and type II diabetic patients responded almost identically to spironolactone therapy with regard to reduction in albuminuria and blood pressure.[11,14]

The rationale for blocking aldosterone in patients with diabetic nephropathy is the findings from recent experimental studies showing that aldosterone induces vascular and glomerular sclerosis, inflammation, and tubular damage independently of angiotensin II levels.[32–34] Aldosterone secretion is mainly regulated by angiotensin II levels, but also by potassium levels and adrenocorticotropic hormone. Blockade of the RAS with an ACEI or an ARB should therefore theoretically reduce plasma levels of aldosterone. An aldosterone escape phenomenon has however been described during treatment with these agents, that is, circulating aldosterone levels increase during RAS-blocking treatment.[12,35] We previously reported that aldosterone escape occurred in 40% of type I diabetic patients with

diabetic nephropathy treated with losartan for 3 years.[29] Presence of aldosterone escape was associated with an enhanced decline in GFR.[29]

In summary, our short-term study suggests that low-dose spironolactone in addition to recommended antihypertensive treatment including ACEIs or ARBs is well tolerated and induces cardiorenal protection, in terms of reduction in albuminuria and blood pressure, in patients with diabetic nephropathy and nephrotic range albuminuria. Special attention should be paid to plasma potassium levels. Further studies are needed to evaluate the long-term renoprotective effect and tolerability of aldosterone antagonism.

MATERIALS AND METHODS
Subjects
From the Steno Diabetes Center, we consecutively enrolled 20 diabetic patients with diabetic nephropathy (17 men and three women). Based on data in our patient registry, we identified 126 patients who had nephrotic range albuminuria. However, only 42 of these patients fulfilled all inclusion criteria for the present study and were invited to participate in the study. Twenty-two of these patients declined participation and 20 patients agreed to participate in the study. Data from 12 of these patients have already been included in previous publications,[11,14] but without any detailed analysis of the impact of spironolactone on nephrotic range albuminuria.

All patients fulfilled the following inclusion criteria: type I or type II diabetes mellitus, diabetic nephropathy, and hypertension (>130/80 mm Hg). Furthermore, all patients had persistent nephrotic range albuminuria (>2500 mg/24 h in three consecutive urine collections) at the time of the screening visit before randomization, despite antihypertensive treatment, including an ACEI or ARB in recommended doses. Diabetic nephropathy was diagnosed clinically if the following criteria were fulfilled: persistent albuminuria, presence of diabetic retinopathy, and the absence of any clinical or laboratory evidence of other kidney or renal tract disease.[36] Kidney biopsies verifying the diagnosis of diabetic glomerulopathy had previously been performed in two patients not having diabetic retinopathy at study start.

Exclusion criteria at entry were GFR < 30 ml/min/1.73 m^2, plasma potassium > 4.5 mmol/l, known renal artery stenosis, age < 18 years, pregnancy, breastfeeding, lack of safe contraception in women, abuse of alcohol or medicine, inability to understand the patient information, and allergy to ACEI, ARB, or spironolactone. Prespecified criteria for termination from the study were severe symptoms of hypotension, systolic blood pressure < 100 mm Hg, increase in plasma creatinine of > 35% within the first 2 weeks of treatment, or plasma potassium > 5.5 mmol/l.

Design
We performed a randomized, double-masked, placebo-controlled, crossover trial consisting of two treatment periods, each lasting 2 months. In random order, patients received spironolactone 25 mg once daily for 2 months and matching placebo tablets once daily for 2 months. There was no drug washout period between treatment periods ('active washout'[37]). The study medication was given in the morning and was added to the patients' ongoing antihypertensive treatment, including a RAS-blocking agent in recommended doses (see the Results section). All patients received diuretics in individual doses before entry into the study to treat and prevent fluid retention

and hyperkalemia. The type and dose of prior antihypertensive treatment, including diuretics, remained unchanged throughout the study.

At the end of each treatment period, the primary end point, albuminuria, and the secondary end points, 24-h blood pressure and GFR, were determined. On the day of GFR determination, patients arrived in a fasting state at 0800, and after 30 min of supine rest the initial blood samples were drawn and breakfast was served. For safety reasons, blood pressure, plasma potassium, plasma sodium, and plasma creatinine were determined 1, 2, and 4 weeks after the beginning of each treatment period.

Randomization was concealed with computer-generated envelopes. The code was not broken until all data were entered into a database, which was locked for editing.

Drug compliance was assessed by tablet counts, and by evaluating the expected compensatory increase in plasma aldosterone and PRA. Dietary intake of protein and salt was not restricted; however, all patients were given written and oral information on how to lower potassium intake in the diet.

The study was performed according to the Declaration of Helsinki Principles and was approved by the ethical committee of Copenhagen County. All patients gave their informed consent.

Laboratory procedures

Albuminuria was determined as the geometric mean of three consecutive 24-h urine collections, completed immediately before the end of each treatment period (Turbidimetry, Hitachi 912 system; Roche Diagnostics, Mannheim, Germany) and fractional clearance of albumin (θ_{Alb}) was determined as (urinary albumin excretion)/(plasma albumin concentration) $\times$ (GFR).

Urinary excretion of sodium, potassium, creatinine, and urea was determined as the mean of three consecutive 24-h urine samples (Hitachi 912 system; Roche Diagnostics).

Blood pressure was measured by a 24-h ABP device (Takeda TM2421; A&D Medical, Tokyo, Japan). Blood pressure was measured every 15 min during the day (0700 to 2300) and every 30 min during the night (2300 to 0700). Values were averaged for each hour before calculating day, night, and 24-h blood pressures. Office blood pressure was measured using an appropriate cuff with a sphygmomanometer after at least 10 min rest in the sitting position. Three readings, 2 min apart and read to the nearest 2 mm Hg, were recorded and the average value was used for calculation.

GFR was measured after a single intravenous injection of 3.7 MBq ^{51}Cr-EDTA at 0830 by determining the radioactivity in venous blood samples taken 180, 200, 220, and 240 min after injection.[38,39] The results were standardized for 1.73 m² body surface area, using the patient's surface area at the start of the study. The mean day-to-day coefficient of variation is 4% in our laboratory.

From venous samples, hemoglobin concentration (Sysmex SF3000, Sysmex Corporation, Kobe, Japan), and plasma potassium, sodium, creatinine, and cholesterol concentrations were determined (Hitachi 912 system; Roche Diagnostics), and HbA$_{1c}$ was measured by high-performance liquid chromatography (normal range: 4.1–6.4%) (Tosoh automated glycohemoglobin analyser; Tosoh Bioscience, Minato, Japan). Blood samples for PRA and aldosterone concentrations were taken after 30 min of supine rest. PRA was measured by a method based on determining by radioimmunoassay the amount of angiotensin I generated, as previously described.[40] Plasma aldosterone was measured using a commercially available radioimmunoassay (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA, USA).

Statistical analysis

For power calculation, we previously calculated the s.d. (log scale 0.1771) of the mean difference in urinary albumin excretion rate in three consecutive 24-h urine samples collected twice within 3 months in 36 patients with diabetic nephropathy. On the basis of these data, a sample-size calculation showed a necessary minimum of 16 patients to detect a 25% change in urinary albumin excretion rate ($\alpha = 0.05$, $\beta = 0.80$).

Normally distributed variables are expressed as means $\pm$ s.d. (Table 1) and means $\pm$ s.e.m. Changes in variables between visits are expressed as means with 95% CI. Albuminuria, PRA, plasma aldosterone, and θ_{Alb} clearance were logarithmically transformed before statistical analysis owing to their skewed distribution and are given as geometric means (95% CI). Changes in these variables during treatment with spironolactone as compared to placebo are expressed in percent. All comparisons of normally or log normally distributed parameters were performed with a paired Student's t-test. Comparison of normally and log normally distributed parameters between two groups (type I vs type II diabetic patients) was performed using an independent samples t-test, whereas a Mann–Whitney test was used for the comparison of urinary sodium excretion owing to lack of normal distribution. Data were tested for a time and carryover effect as described by Altman.[41]

Linear regression analysis was used to analyze for correlations between the relative change in albuminuria and changes in arterial blood pressure, GFR, plasma aldosterone levels, and PRA.

$P < 0.05$ was considered significant (two-tailed test). Data were evaluated using SPSS version 13.0 (SPSS, Chicago, IL, USA).

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