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Effects of sodium restriction and hydrochlorothiazide on RAAS blockade efficacy in diabetic nephropathy: a randomised clinical trial

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Summary

Background **Reduction of dietary sodium intake or diuretic treatment increases renin-angiotensin-aldosterone system (RAAS) blockade efficacy in non-diabetic nephropathy. We aimed to investigate the effect of sodium restriction and the diuretic hydrochlorothiazide, separately and in combination, added to RAAS blockade on residual albuminuria in patients with type 2 diabetic nephropathy.**

Methods **In this multicentre, double-blind, placebo-controlled, crossover randomised trial, we included patients with type 2 diabetic nephropathy. Main entry criteria were microalbuminaria or macroalbuminuria, and creatinine clearance of 30 mL/min or higher with less than 6 mL/min decline in the previous year. We tested the separate and combined effects of sodium restriction (dietary counselling in the outpatient setting) and hydrochlorothiazide (50 mg daily), added to standardised maximal angiotensin-converting enzyme (ACE) inhibition (lisinopril 40 mg daily), on albuminuria (primary endpoint). Patients were given hydrochlorothiazide (50 mg per day) or placebo during four treatment periods of 6 weeks. Both treatments were combined with regular sodium diet or sodium restriction (target sodium intake 50 mmol Na^+ per day). The 6-week treatment periods were done consecutively in a random order. Patients were randomised in blocks of two patients. The trial was analysed by intention to treat. The trial is registered with TrialRegister.nl, number 2366.**

Findings **Of 89 eligible patients, 45 were included in the study. Both sodium restriction and hydrochlorothiazide significantly reduced albuminuria, irrespective of treatment sequence. Residual geometric mean albuminuria with baseline treatment was 711 mg per day (95% CI 485–1043); it was significantly reduced by sodium restriction (393 mg per day [258–599], p=0·0002), by hydrochlorothiazide (434 mg per day [306–618], p=0·0003), and to the greatest extent by their combination (306 mg per day [203–461], p<0·0001). Orthostatic complaints were present in two patients (4%) during baseline treatment, five (11%) during addition of sodium restriction, five (11%) during hydrochlorothiazide treatment, and 12 patients (27%) during combination treatment. No serious adverse events occurred.**

Interpretation **We conclude that sodium restriction is an effective non-pharmacological intervention to increase RAAS blockade efficacy in type 2 diabetic nephropathy.**

Funding **None.**

Introduction

The combination of diabetes and nephropathy is associated with high morbidity and mortality.[1] Albuminuria and hypertension substantially contribute to progression of renal and cardiovascular disease.[2] Reduction of albuminuria and blood pressure by pharmacological blockade of the renin-angiotensin-aldosterone system (RAAS) can attenuate this increased risk, and is regarded as the cornerstone of treatment in diabetic nephropathy.[3]

Unfortunately, cardiovascular and renal protection by RAAS blockade is far from complete, with a high residual risk despite treatment amounting to an event rate of 8% per year.[3] Therefore, strategies to improve the efficacy of RAAS blockade could be useful. In patients with non-diabetic nephropathy, dietary sodium restriction or diuretic treatment, or both, can potentiate RAAS blockade efficacy.[4,5] In patients with diabetes, sodium supplementation blunts the efficacy of RAAS blockade,[6] suggesting that sodium restriction might be useful in patients with diabetic nephropathy as well. Moreover, results from secondary analyses of a randomised trial showed an improved long-term outcome of RAAS blockade in diabetic nephropathy in patients consuming less sodium.[7] So far, however, no controlled studies have been done on the effect of sodium restriction or diuretic treatment on RAAS blockade efficacy in diabetic nephropathy. We therefore investigated, in a randomised rotation design, the effect of sodium restriction and the diuretic hydrochlorothiazide, and their combination, added to RAAS blockade with angiotensin-converting enzyme (ACE) inhibitors, on residual albuminuria and blood pressure in patients with type 2 diabetic nephropathy. To assess the representativeness for clinical practice of the unrestricted sodium intake in the present trial, we also measured urinary sodium excretion in a larger unselected sample of outpatients from the same recruitment setting.

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Methods

Trial design and participants

This multicentre, double-blind, placebo-controlled, crossover randomised clinical trial was done by the Holland Nephrology Study (HONEST) Group between Oct 10, 2009, and Dec 10, 2012, at the Departments of Internal Medicine or Divisions of Nephrology of three medical centres (University Medical Center Groningen, ZGT Hospital Almelo, and Medical Center Leeuwarden) in the Netherlands. This trial is in accordance with the Declaration of Helsinki, approved by the independent medical ethics committee of the University Medical Center of the University of Groningen (identification number 2010/228), and has been done according to the guidelines for Good Clinical Practice. All participants provided written informed consent before entry into the trial. A data safety monitoring board was not appointed.

We screened consecutive patients with type 2 diabetes at outpatient clinics for the presence of diabetic nephropathy, as diagnosed by the patient's own nephrologist on the basis of medical history and analysis of blood and urine. Presence of albuminuria (defined as albuminuria >30 mg per day or urinary albumin concentration >20 mg/L or urinary albumin:creatinine ratio >2·5 mg/mmol for men and >3·5 mg/mmol for women) at time of screening and after completion of the run-in period was the main inclusion criterion. Other inclusion criteria were age 18 years or older, and creatinine clearance of 30 mL/min or higher with a less than 6 mL/min decline in the previous year. For safety reasons, we excluded patients with a systolic blood pressure of 180 mm Hg or higher, a diastolic blood pressure of 110 mm Hg or higher, or overt nephrotic syndrome at baseline, because these disorders might need more aggressive therapy during the timeframe of the trial. Other exclusion criteria were a second primary renal disease in addition to diabetic nephropathy, type 1 diabetes, renovascular hypertension, a cardiovascular or cerebrovascular event within 3 months before inclusion, serum potassium of 6·0 mmol/L or higher, transplantation or immunosuppressive treatment, contraindication for the use of lisinopril or hydrochlorothiazide, pregnancy or lactation, incompliance to medication, and inability to provide informed consent.

To assess the representativeness of sodium intake in our trial setting for the general outpatient setting, we analysed data for 24 h sodium excretion in a larger sample of unselected outpatients with type 2 diabetic nephropathy in the same outpatient setting as used for recruitment. For direct comparison of sodium intake with the trial population, we selected patients matched for age and sex, which are determinants of sodium intake.

Randomisation and masking

The drug intervention was double blind, whereas the dietary intervention was open label. An independent pharmacist used a computer program to randomise patients in blocks of two. No stratification was needed as the trial has a crossover design. An independent pharmacist randomised treatment sequences. Patients were sequentially enrolled according to moment of recruitment. The randomisation code remained secret during the entire trial. All patients, investigators, and health-care providers were masked, apart from the pharmacist who did the randomisation. On completion of the trial, the principal investigator (AJK) provided the pharmacist and the medical ethics committee with a written statement that the trial was completed, after which masking ended.

Procedures

Figure 1 shows a schematic overview of the trial design. During a run-in period of 6 weeks, patients were titrated to maximum dose of ACE inhibitor (lisinopril 40 mg per day), whereas all other RAAS blockers and diuretics were discontinued. Maximum dose ACE inhibition served as background treatment and was kept stable throughout the trial. Additional antihypertensive drugs, such as α blockers, β blockers, and calcium-channel blockers, were allowed when dosage was stable throughout the trial. No dietary intervention took place during the run-in period.

After the run-in period, patients were given hydrochlorothiazide at maximum dose (50 mg per day) or placebo during four consecutive treatment periods of 6 weeks. Both treatments were combined with, consecutively, regular sodium diet or sodium restriction (target sodium intake 50 mmol Na^+ per day [equal to about 1200 mg Na^+ per day or 3 g NaCl per day]). Use of 6-week treatment periods was based on previous studies from our department in patients with non-diabetic nephropathy, showing that changes in albuminuria generally stabilise 3–4 weeks after the change in therapy.[4]

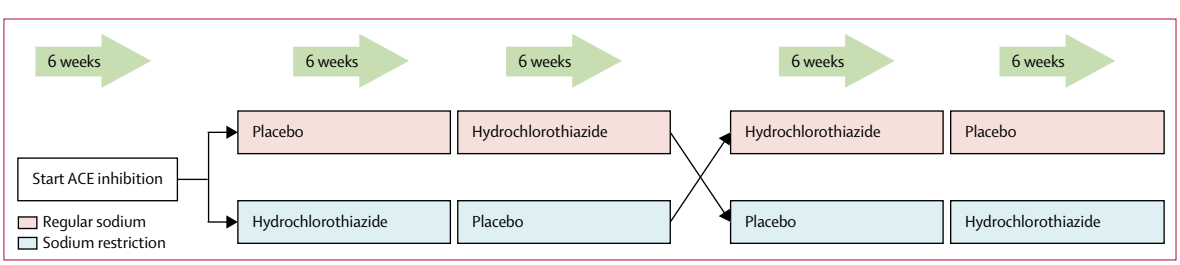

Figure 1: **Trial design**

To prevent systematic errors resulting from the crossover design, the different treatment periods were done in random order. The trial protocol (appendix) did not include a washout period between treatment periods because of the randomisation procedure and the short half-life of both interventions (hydrochlorothiazide 9·5–26 h depending on extent of renal impairment; sodium restriction <1 week;[8] background therapy, lisinopril 12·6 h). All patients received a list of food products that are commonly consumed in the Netherlands, together with their sodium content, at the time of inclusion. Professional dietitians gave further dietary counselling. Except for a request to achieve the particular sodium targets (ie, 50 mmol Na^+ per day during sodium restriction and 200 mmol Na^+ per day during the regular sodium diet), dietitians did not receive extra training or a script for this trial.

Every patient had one or two dietary counselling sessions. Individualised counselling used the general principle of remaining as close as possible to the patients' preferences and nutritional habits, to increase feasibility and compliance, taking into account adequacy of nutritional requirements as well as sodium content. For the periods on the regular sodium diet, patients were advised to maintain habits regarding sodium intake. For the periods on sodium restriction, patients were advised not to add any salt to their food and to replace sodium-rich products with sodium-poor products. Compliance to sodium restriction was monitored by measuring urinary sodium excretion in 24 h urine samples in the middle and at the end of each 6-week treatment period. Compliance to study medication was assessed by pill counts. Patients received extensive feedback on their sodium intake every 3 weeks, either by telephone or during the visit of the patient to the outpatient clinic from the principal investigator.

Patients visited the outpatient clinic at the end of every treatment period for clinical assessment, blood pressure measurement, and venous blood sampling. Albuminuria was assessed in 24 h urine samples that patients collected 1 or 2 days before the hospital visit. Albuminuria was measured with a turbidimetric assay using benzethonium chloride (Modular, Roche Diagnostics, Mannheim, Germany), in a single batch. Blood pressure was measured at 1-min intervals with a semiautomatic device (Dinamap, GE Medical Systems, Milwaukee, WI, USA) with the patient being in a semisupine position. After 15 min of measurements, we discarded the last blood pressure measurement to avoid confounding and used the mean of the second-to-last four readings for analysis. Blood electrolytes, lipids, and proteins, and urinary electrolytes were determined by an automated multi analyser (Modular, Roche Diagnostics, Mannheim, Germany). Creatinine clearance was calculated from creatinine concentrations in plasma and 24 h urine samples. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI ((Chronic Kidney Disease Epidemiology Collaboration) equation.

Outcomes

The primary outcome was albuminuria, measured after each 6-week intervention. Secondary outcomes were blood pressure (systolic and diastolic) and renal function (creatinine clearance, eGFR, and serum creatinine). Extracellular volume was also prespecified as a secondary outcome measure; however, measurement of extracellular volume could be done only in a subset of the patients, and for this reason is not reported. Other response measures were bodyweight; serum sodium, potassium, urea, albumin, glucose, and total cholesterol; urinary excretion of protein, urea, and potassium; and urinary albumin:creatinine excretion ratio and protein:creatinine excretion ratio.

See Online for appendix

Statistical analysis

We expected patients to present with about 120 mg albuminuria per day with baseline treatment (while on ACE inhibition and regular sodium intake). We did the sample size calculation on the basis of a hypothesised 20% reduction in albuminuria by addition of sodium restriction, which corresponds to a 0·22 reduction on a log-transformed scale. From these numbers, assuming an effect size of 0·5, we estimated that 45 patients had to complete the crossover design to provide 90% power with an α of 0·05 to detect a significant difference. The sample size is smaller than needed in a non-crossover trial because the same patient provides data for every treatment, and thereby increases power owing to within-patient variability being smaller than between-group variability.[9,10]

We did intention-to-treat analyses after locking of the database. Skewed variables were $\log_{10}$-transformed before statistical testing. For the primary outcome, we analysed data by linear mixed model analysis, including a Bonferroni correction, with $\log_{10}$-transformed albuminuria as the dependent variable, patients as a random factor, and treatments and sequences as well as their interactions (treatment × sequence) as fixed factors, thus allowing estimation of effects of treatment sequence and detection of adjusted effects of the different treatment regimens. We used *t* tests to provide data on the differential effects of sodium restriction and hydrochlorothiazide on albuminuria and on other outcome measures.

We did six comparisons for every parameter: (1) ACE inhibition plus regular sodium plus placebo *vs* ACE inhibition plus sodium restriction plus placebo; (2) ACE inhibition plus sodium restriction plus placebo *vs* ACE inhibition plus regular sodium plus hydrochlorothiazide; (3) ACE inhibition plus regular sodium plus hydrochlorothiazide *vs* ACE inhibition plus sodium restriction plus hydrochlorothiazide; (4) ACE inhibition plus regular sodium plus placebo *vs* ACE inhibition plus regular sodium plus hydrochlorothiazide; (5) ACE inhibition plus sodium restriction plus placebo *vs* ACE inhibition plus sodium restriction plus

hydrochlorothiazide; and (6) ACE inhibition plus regular sodium plus placebo *vs* ACE inhibition plus sodium restriction plus hydrochlorothiazide.

To test for the nominal difference in efficacy between sodium restriction and hydrochlorothiazide, and their combination, on albuminuria and blood pressure, we calculated relative reduction from baseline treatment with subsequent statistical testing using paired *t* tests. We did three comparisons for this analysis: (1) relative reduction by sodium restriction from baseline treatment *vs* relative reduction by hydrochlorothiazide from baseline treatment; (2) relative reduction by sodium restriction from baseline treatment *vs* relative reduction by the combination sodium restriction and hydrochlorothiazide from baseline; and (3) relative reduction by hydrochlorothiazide from baseline treatment *vs* relative reduction by the combination sodium restriction and hydrochlorothiazide from baseline treatment. Comparisons between the trial cohort and the reference cohort were tested with ANOVA.

We present data as mean (SD) when normally distributed, or as geometric mean (95% CI) or median (IQR) when non-normally distributed. We used SPSS 20 for Windows and GraphPad Prism version 5 for analyses. Two-sided p values of less than 0·05 were deemed significant.

Role of sponsor and funding source

The sponsor of this trial is the University Medical Center Groningen, Groningen, the Netherlands. The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to the data. The corresponding author had the final responsibility for the decision to submit for publication. No funding was received for this trial.

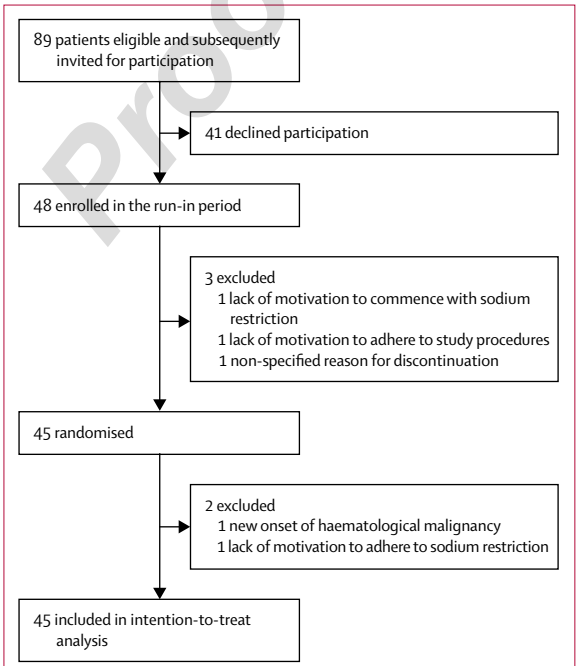

Figure 2: **Trial profile**

Results

Of 89 patients with type 2 diabetic nephropathy assessed for eligibility, 45 patients gave written informed consent and were subsequently enrolled in the run-in period (figure 2). Table 1 shows the characteristics of these 45 patients. Time since diagnosis of diabetes was 9 years (IQR 5–19) and about half the study population had established macrovascular disease. Mean HbA_{1c} was 7·1% (SD 0·9), indicating adequate glycaemic control by insulin treatment, biguanide, or sulfonylurea derivative (or a combination). In addition to standardised background ACE inhibition, 37 (82%) of the 45 patients received at least one antihypertensive drug (range 1–3), either α blockade, β blockade, or calcium-channel blockade.

We also included a larger sample of 255 unselected outpatients with type 2 diabetic nephropathy that we selected to assess the representativeness of sodium intake in our trial setting. Mean age of this reference population was 63 years (SD 9), 137 (54%) were men, mean BMI was 33 kg/m² (SD 6), and mean creatinine clearance was 109 mL/min (SD 45). In the age-matched

	All patients (n=45)
Age, years (mean, SD)	65 (9)
Male sex	38 (84%)
White ethnic origin	45 (100%)
BMI, kg/m² (mean, SD)	32 (5)
Creatinine clearance, mL/min (mean, SD)	101 (47)
eGFR, mL/min per 1·73 m² (mean, SD)	65 (25)
Albuminuria, mg per day (geometric mean, 95% CI)	711 (485–1043)
Diabetes duration, years (median, IQR)	9 (5–19)
Macrovascular disease	21 (47%)
Non-trial antihypertensive medication (in addition to background ACE inhibition)	
0	8 (18%)
1	17 (38%)
2	17 (38%)
3	3 (6%)
Type of non-trial antihypertensive medication	
α blockade	4 (9%)
β blockade	27 (45%)
Calcium-channel blockade	27 (45%)
Diabetes medication	
Insulin	25 (53%)
Biguanide	32 (71%)
Sulfonylurea derivative	17 (38%)
HbA_{1c}, % (mean, SD)	7·1% (0·9)

Data are number of patients (%) unless otherwise indicated. eGFR=estimated glomerular filtration rate. ACE=angiotensin converting enzyme.

Table 1: **Patient characteristics**

and sex-matched reference population (n=160), mean age was 65 years (SD 8), 136 (85%) were male, mean BMI was 32 kg/m² (SD 5), and creatinine clearance was 110 mL/min (SD 46; all p>0·05 *vs* trial population).

We used urinary sodium excretion as a measure of dietary sodium intake. Sodium excretion significantly decreased from 224 mmol (SD 73) per day during the regular sodium diet to 148 mmol (SD 65) per day during sodium restriction when combined with placebo and 164 mmol (SD 73) per day with hydrochlorothiazide (both p<0·0001 *vs* regular sodium intake; figure 3). In the reference population of unselected outpatients, urinary sodium excretion was 189 mmol (SD 80) per day. In the age-matched and sex-matched reference population, the mean urinary sodium excretion was 207 mmol (SD 79) per day—ie, similar to that of the trial population (p=0·20) during regular sodium intake.

Overall compliance to hydrochlorothiazide (ie, the proportion of pills taken out of all provided pills) was 97%. All patients had good compliance (>80% of hydrochlorothiazide and placebo tablets taken during all four treatment periods), apart from four patients during placebo (one for regular sodium, three for sodium restriction) and three patients during hydrochlorothiazide (two for regular sodium, one for sodium restriction). Of these patients, only one had incompliance in more than one treatment period (this patient had a compliance of 70–80% during regular sodium diet plus placebo, sodium restriction plus placebo, and regular sodium plus hydrochlorothiazide periods).

Albuminuria values, in the 43 patients who completed the regular sodium period, were available from all 43 patients during diuretic intervention and from 42 during placebo. Albuminuria values, in the 45 patients who completed the sodium restriction period, were available from 39 patients during diuretic intervention and from 42 during placebo.

We analysed changes in albuminuria using linear mixed model analyses to account for repeated measurements and to check for presence of any potential carry-over effect of treatment. This analysis showed that treatment with sodium restriction and hydrochlorothiazide significantly reduced albuminuria, independent of treatment sequence (table 2; figure 4). Reduction in albuminuria did not differ significantly between sodium restriction and hydrochlorothiazide treatments (p=0·79). The combination of sodium restriction and hydrochlorothiazide reduced albuminuria further than either treatment alone (p<0·0001 *vs* baseline treatment). The percent reduction in albuminuria was similar for sodium restriction (42% [95% CI 24–55]) and hydrochlorothiazide (42% [23–55], p=0·79 for difference between treatments), and was larger for their combination (61% [45–71], p=0·0018 for both). 14 (33%) of 43 patients had an albuminuria value below 300 mg/day with baseline treatment; this percentage increased to 49% (19/39) in response to sodium restriction, 43% (18/42) in response to hydrochlorothiazide, and 52% (22/42) with their combination. Results were similar for creatinine-adjusted albuminuria, and for crude and creatinine-adjusted proteinuria (table 2).

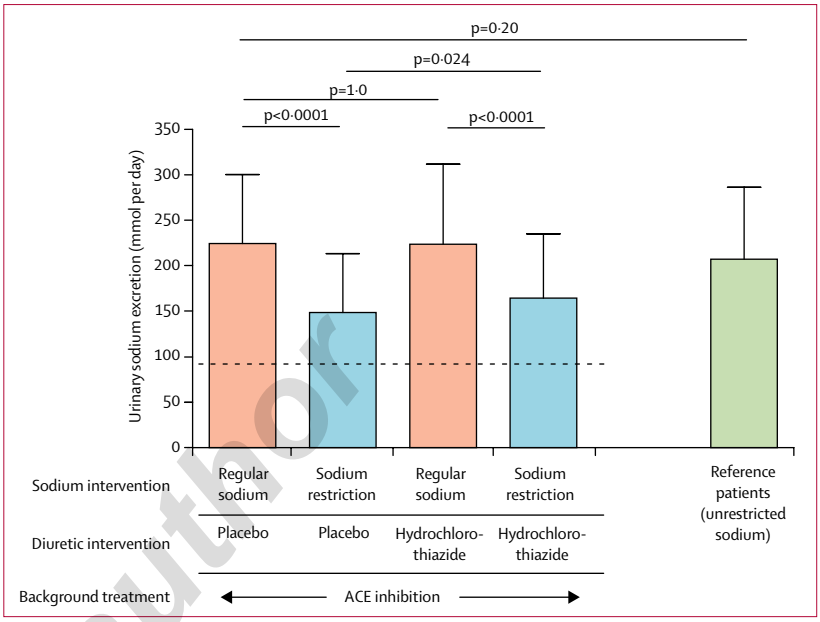

Figure 3: **Urinary sodium excretion in trial patients (n=45) during regular sodium diet and sodium restriction (during placebo and hydrochlorothiazide), and in age-matched and sex-matched reference patients with diabetic nephropathy (n=160) during unrestricted sodium**
Data are shown as mean (SD). Dotted line represents sodium intake (85 mmol Na^+ per day) recommended for the general population by WHO.[11]

We also measured systolic and diastolic blood pressure in response to sodium restriction and hydrochlorothiazide, and their combination, during ACE inhibition (figure 5, table 2). In comparison with baseline treatment, mean systolic blood pressure was significantly reduced by sodium restriction (change –5·3 mm Hg [95% CI –1·5 to –9·1], p=0·0080), by hydrochlorothiazide (change –12·0 mm Hg [–7·8 to –15·2], p<0·0001), and to the greatest extent by their combination (change –17·0 mm Hg [–13·1 to –20·9], p<0·0001). The percent reduction in systolic blood pressure was significantly larger for hydrochlorothiazide than for sodium restriction (difference in relative response 4·3% [95% CI 1·0–7·5], p=0·012) and even larger with their combination than with either sodium restriction alone (difference in relative response 8·0% [4·9–10·9], p<0·0001) or hydrochlorothiazide alone (difference in relative response 3·5% [0·8–6·1], p=0·012). Eight (19%) of 43 patients had adequate systolic blood pressure control with baseline treatment (≤130 mm Hg); this increased to 24% (11/45) in response to sodium restriction, 40% (17/43) in response to hydrochlorothiazide, and 62% (28/45) with their combination.

Similarly, in comparison with baseline treatment, mean diastolic blood pressure was decreased by sodium restriction (change –3·4 mm Hg [95% CI –1·0 to –5·8], p=0·0067), with hydrochlorothiazide (change –6·3 mm Hg [–4·0 to –8·6], p<0·0001), and to the

greatest extent with their combination (change –9·5 mm Hg [–7·1 to –11·9], p<0·0001). Percent reductions in diastolic blood pressure did not differ significantly between sodium restriction and hydrochlorothiazide (difference in relative response 3·4% [95% CI –0·07 to 6·9], p=0·055), although the reduction in response to combined treatment was significantly larger than either sodium restriction alone (difference in relative response 7·3% [3·8–10·7], p=0·0001) or hydrochlorothiazide alone (difference in relative response 3·9% [1·1–6·6], p=0·007). 23 (53%) of 43 patients had adequate diastolic blood pressure control

	ACE inhibition		Effect of sodium restriction	Difference in effect between sodium restriction and hydrochlorothiazide	Difference in effect of maximal treatment *vs* baseline treatment
	Regular sodium diet	Sodium restriction			
Clinical measurements					
Systolic blood pressure (mm Hg)				0·0096	<0·0001
Placebo	147 (16)	141 (16)	0·0080	..	..
Hydrochlorothiazide	135 (16)	129 (14)	0·0009	..	..
Effect of hydrochlorothiazide	<0·0001	<0·0001	..	..	..
Diastolic blood pressure (mm Hg)				0·036	<0·0001
Placebo	82 (10)	79 (10)	0·0067	..	..
Hydrochlorothiazide	76 (9)	72 (8)	0·0055	..	..
Effect of hydrochlorothiazide	<0·0001	<0·0001	..	..	..
Mean arterial pressure (mm Hg)				0·017	<0·0001
Placebo	104 (11)	100 (11)	0·0055	..	..
Hydrochlorothiazide	96 (10)	91 (10)	0·0012	..	..
Effect of hydrochlorothiazide	<0·0001	<0·0001	..	..	..
Bodyweight (kg)				0·95	<0·0001
Placebo	102 (18)	100 (18)	<0·0001	..	..
Hydrochlorothiazide	100 (18)	98 (18)	0·0027	..	..
Effect of hydrochlorothiazide	<0·0001	0·0002	..	..	..
Serum measurements					
Sodium (mmol/L)				0·57	<0·0001
Placebo	140·3 (3·1)	139·7 (3·3)	0·058	..	..
Hydrochlorothiazide	139·6 (3·2)	138·0 (3·7)	0·0007	..	..
Effect of hydrochlorothiazide	0·018	0·010	..	..	..
Potassium (mmol/L)				0·0053	0·97
Placebo	4·4 (0·4)	4·5 (0·5)	0·025	..	..
Hydrochlorothiazide	4·3 (0·5)	4·5 (0·5)	0·065	..	..
Effect of hydrochlorothiazide	0·18	0·29	..	..	..
Urea (mmol/L)				0·047	<0·0001
Placebo	8·5 (3·1)	9·2 (4·4)	0·053	..	..
Hydrochlorothiazide	10·1 (3·8)	11·3 (5·5)	0·024	..	..
Effect of hydrochlorothiazide	<0·0001	<0·0001	..	..	..
Creatinine clearance (mL/min)				0·56	0·0017
Placebo	101 (47)	99 (48)	0·48	..	..
Hydrochlorothiazide	97 (47)	89 (42)	0·0026	..	..
Effect of hydrochlorothiazide	0·37	0·0022	..	..	..
eGFR (mL/min per 1·73 m^2)				0·0094	<0·0001
Placebo	65 (25)	65 (27)	0·63	..	..
Hydrochlorothiazide	60 (27)	59 (25)	0·042	..	..
Effect of hydrochlorothiazide	0·0034	0·0001	..	..	..
Creatinine (μmol/L)				0·015	<0·0001
Placebo	111 (41)	114 (51)	0·49	..	..
Hydrochlorothiazide	122 (47)	125 (52)	0·17	..	..
Effect of hydrochlorothiazide	0·0003	0·0028	..	..	..

(Table 2 continues on next page)

	ACE inhibition		Effect of sodium restriction	Difference in effect between sodium restriction and hydrochlorothiazide	Difference in effect of maximal treatment *vs* baseline treatment
	Regular sodium diet	Sodium restriction			
(Continued from previous page)					
Albumin (g/L)				0·88	<0·0001
Placebo	37·7 (3·8)	38·2 (3·9)	0·027	..	..
Hydrochlorothiazide	38·2 (3·7)	39·0 (3·8)	0·0031	..	..
Effect of hydrochlorothiazide	0·0035	0·039	..	..	..
Glucose (mmol/L)				0·18	0·61
Placebo	8·7 (3·9)	8·8 (4·0)	0·79	..	..
Hydrochlorothiazide	9·4 (3·8)	8·9 (4·4)	0·41	..	..
Effect of hydrochlorothiazide	0·28	0·72	..	..	..
Total cholesterol (mmol/L)				0·68	0·043
Placebo	4·6 (1·4)	4·5 (1·5)	0·88	..	..
Hydrochlorothiazide	4·5 (1·2)	4·1 (1·1)	0·029	..	..
Effect of hydrochlorothiazide	0·33	0·040	..	..	..
Urine measurements					
Albumin excretion (mg/day)				0·79	<0·0001
Placebo	711 (485–1043)	393 (258–599)	0·0002	..	..
Hydrochlorothiazide	434 (306–618)	306 (203–461)	0·0018	..	..
Effect of hydrochlorothiazide	0·0003	0·0008	..	..	..
Albumin:creatinine excretion (mg/mmol)				0·48	<0·0001
Placebo	52 (35–77)	29 (19–45)	0·0004	..	..
Hydrochlorothiazide	31 (21–44)	23 (15–34)	0·012	..	..
Effect of hydrochlorothiazide	<0·0001	0·0010	..	..	..
Protein excretion (g/day)				0·53	<0·0001
Placebo	1·3 (0·9–1·8)	0·9 (0·6–1·2)	<0·0001	..	..
Hydrochlorothiazide	0·8 (0·6–1·1)	0·6 (0·4–0·8)	0·0005	..	..
Effect of hydrochlorothiazide	<0·0001	0·0001	..	..	..
Protein:creatinine excretion (mg/mmol)				0·16	<0·0001
Placebo	0·09 (0·06–0·13)	0·07 (0·05–0·09)	<0·0001	..	..
Hydrochlorothiazide	0·06 (0·04–0·08)	0·04 (0·03–0·06)	0·017	..	..
Effect of hydrochlorothiazide	<0·0001	0·0005	..	..	..
Urea excretion (mmol/day)				0·35	0·84
Placebo	373 (332–421)	342 (303–387)	0·10	..	..
Hydrochlorothiazide	367 (329–411)	337 (303–376)	0·15	..	..
Effect of hydrochlorothiazide	0·14	0·64	..	..	..
Potassium excretion (mmol/day)				0·77	0·30
Placebo	74 (67–82)	77 (68–86)	0·31	..	..
Hydrochlorothiazide	76 (69–84)	72 (66–79)	0·11	..	..
Effect of hydrochlorothiazide	0·62	0·092	..	..	..

Data are mean (SD) or geometric mean (95% CI). Effect of sodium restriction: sodium restriction *vs* regular sodium on same treatment. Effect of hydrochlorothiazide: hydrochlorothiazide *vs* placebo on the same diet. Difference in effect between sodium restriction and hydrochlorothiazide: hydrochlorothiazide *vs* sodium restriction. Difference in effect of maximal treatment *vs* baseline treatment: combination of sodium restriction and hydrochlorothiazide *vs* baseline treatment. ACE=angiotensin-converting enzyme. eGFR=estimated glomerular filtration rate.

Table 2: **The effect on clinical and biochemical measurements of sodium restriction, hydrochlorothiazide, and their combination, added to ACE inhibition**

with baseline treatment (≤80 mm Hg); this percentage was 47% (21/45) in response to sodium restriction, 67% (29/43) in response to hydrochlorothiazide, and 84% (38/45) with their combination.

We noted similar changes to those observed for systolic and diastolic blood pressure for mean arterial blood pressure in response to sodium restriction, hydrochlorothiazide, and their combination (table 2).

Renal function as measured by creatinine clearance was preserved during ACE inhibition and regular sodium and remained unaffected by sodium restriction (table 2; change 3 mL/min [95% CI −5 to 10], p=0·48) and

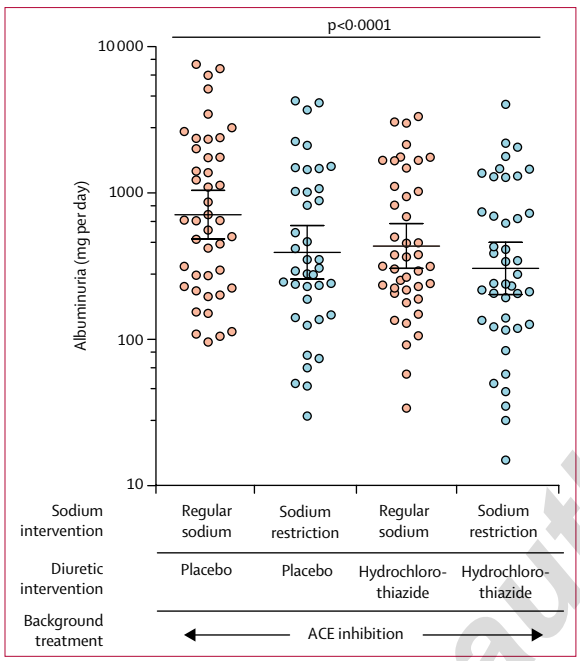

Figure 4: **Effect on albuminuria of sodium restriction, hydrochlorothiazide, and their combination, added to ACE inhibition**
Data are shown as geometric mean (95% CI). p value shows treatment effect by linear mixed modelling.

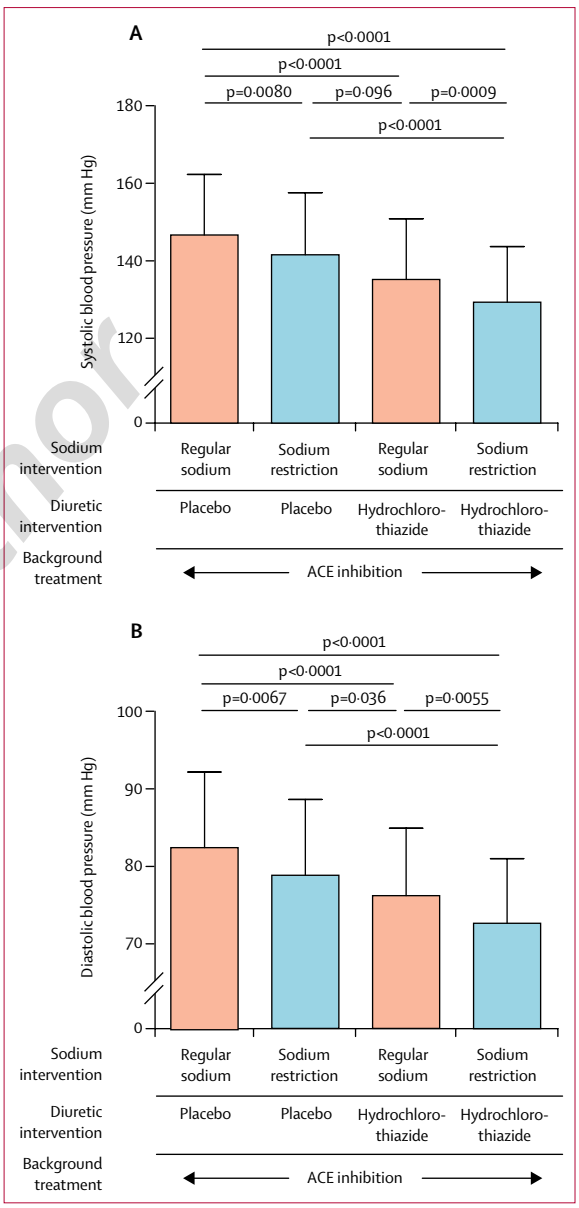

Figure 5: **Effect on systolic and diastolic blood pressure of sodium restriction, hydrochlorothiazide, and their combination, added to ACE inhibition**
Data are shown as mean (SD).

hydrochlorothiazide (change 4 mL/min [–5 to 13], p=0·37); however, creatinine clearance was significantly reduced *vs* baseline treatment by their combination (change –14 mL/min [95% CI –6 to –22], p=0·0017). This decrease was reversible on discontinuation of sodium restriction and hydrochlorothiazide. Table 2 shows data for eGFR and serum creatinine.

Sodium restriction and hydrochlorothiazide had small effects on serum electrolytes without exceeding local laboratory reference values (table 2). Plasma glucose concentrations remained stable throughout the trial, excluding a possible effect of hyperglycaemia on main outcome variables. Furthermore, urinary urea and potassium excretion remained stable during all trial periods, underscoring the specificity of the dietary sodium restriction intervention. Bodyweight during baseline treatment was reduced in response to sodium restriction (table 2; change –1·7 kg [95% CI –0·9 to –2·5], p<0·0001), hydrochlorothiazide (change –1·7 kg [–0·9 to –2·5], p<0·0001), and to the greatest extent by their combination (change –2·9 kg [–2·0 to –3·7], p<0·0001).

Orthostatic complaints were present in two (4%) of 45 patients during baseline treatment, five (11%) during sodium restriction, five (11%) during hydrochlorothiazide treatment, and 12 (27%) with their combination. No serious adverse events were recorded.

Discussion

This trial is the first head-to-head comparison of the effects of sodium restriction and hydrochlorothiazide, and their combination, on the efficacy of RAAS blockade in type 2 diabetic nephropathy. Although sodium excretion remained above recommended values[11] during the sodium restriction periods, sodium restriction or hydrochlorothiazide reduced albuminuria and blood pressure when added to RAAS blockade, and their combination reduced these variables even further. Thus, sodium restriction increases the efficacy of RAAS blockade in diabetic nephropathy even when the restricted sodium intake is high according to current standards. Our findings add to knowledge on the role of sodium restriction in renoprotective efficacy of RAAS blockade (panel).

The albuminuria-lowering efficacy of sodium restriction added to RAAS blockade noted in this study is in line with previous studies in non-diabetic nephropathy,[4,5] hypertension,[12,13] and healthy individuals.[8] In diabetes, only a few randomised studies are available. Sodium restriction lowered urinary albumin or protein excretion, or both, in some[14,15] but not all studies.[16] Of note, these studies were all very small or not primarily designed to study albuminuria, as they also included normo-albuminuric patients.[15,16] Moreover, in previous studies, sodium restriction was investigated without cotreatment with RAAS blockade,[15,16] the current evidence-based standard therapy for diabetic nephropathy.[17] The absence of good quality data in diabetic nephropathy on the effect of sodium restriction on albuminuria is portrayed by the fact that a recent meta-analysis could not address this topic because of insufficient data.[18]

The reduction in blood pressure by sodium restriction during RAAS blockade, as noted here, is also in line with previous data.[4,12,13,19] In diabetes, sodium restriction reduced blood pressure in all[14,20–22] but one study,[23] irrespective of presence of nephropathy, with more prominent effects during concomitant RAAS blockade.[14] Pooled data on sodium restriction in patients with diabetes showed reductions of –7% for systolic blood pressure and –3% for diastolic blood pressure.[18]

We designed and did our trial in line with regular outpatient care for this population to ensure the relevance of our trial for clinical practice. Dietary counselling, particularly, was within the limits of what is feasible in regular outpatient care for these patients in the Netherlands. In this setting, patients could reduce their sodium intake by about 80 mmol per day. Due to high baseline intake this resulted in sodium intake that was still considerably above recommended intake of 85 mmol Na^+ per day, as advised by WHO for the general population,[11] during restricted periods. Of note, the reduction in sodium intake achieved here roughly corresponds to the range of sodium intake studied retrospectively for its association with long-term outcome of RAAS blockade in the diabetes population of the RENAAL and IDNT trials,[7] in which differences of 180–210 mmol per day were associated with a difference of about 50% in renal and cardiovascular endpoints during a follow-up of 4 years. High baseline sodium intake during the trial was apparently not a study artifact. We considered this possibility since sodium intake was not blinded, which could have led to higher sodium intake in patients aware that they were in an unrestricted sodium treatment period. However, we found that sodium intake was similarly high in matched individuals from a larger reference population.

Observational data in non-diabetic renal disease showed more effective protection against progressive loss of renal function with RAAS blockade in patients consuming less sodium.[24] However, recent epidemiological data raised concern on the safety of dietary sodium restriction in diabetes without (advanced) nephropathy, showing either a J curve or an inverse association between sodium intake and outcome in diabetes, with worse outcomes at very low sodium intake.[25,26] Our data do not refute the potential risks of very low sodium intake, but they allow a different perspective, by showing that avoiding excessive sodium intake, as is apparently habitual in this population of patients with type 2 diabetic nephropathy, has substantial potential to improve outcome.

Limited data are available on the effect of the addition of a diuretic on top of RAAS blockade in diabetes.[6] In our trial, in line with previous head-to-head comparisons in non-diabetic nephropathy, the effects of sodium restriction and hydrochlorothiazide on albuminuria were similar.[4,5] However, in our study the effect of hydrochlorothiazide on blood pressure was somewhat larger than the effect of sodium restriction. The maximum changes in albuminuria and blood pressure were obtained by a combination of sodium restriction and hydrochlorothiazide, again in line with data in non-diabetic nephropathy.[4,5] However, combination treatment was associated with a reversible reduction in creatinine clearance. The clinical significance of such a decrease in renal function should be considered. Volume deficit can elicit acute kidney injury, especially during RAAS blockade, but the reduction noted here was small and reversible. Moreover, a slight reduction in renal function during renoprotective treatment has been shown to predict a more favourable renal outcome in the long term, possibly because it indicates amelioration of glomerular hypertension.[27,28] Our data do not support a role of the so-called sodium paradox in this clinical setting of diabetes and nephropathy.[29] This phenomenon—a rise in filtration pressure and glomerular filtration rate during

***Panel*: Research in context**

Systematic review

We searched the scientific literature in December, 2013, for articles published in English, with the search terms "sodium restriction", "salt restriction", "albuminuria", "diabetes", "thiazide", and "diuretic". No large prospective randomised clinical trial on the effect of sodium restriction alone, or in combination with diuretic treatment, on the efficacy of RAAS blockade in diabetic nephropathy was available. In non-diabetic renal disease, salt restriction to 5–6 g per day improves the effects of RAAS blockade on proteinuria and blood pressure.[4,5] In diabetic renal disease, systematic review and meta-analysis showed a possible beneficial effect of sodium restriction.[17] For long-term outcome, more effective protection against progressive renal function loss by RAAS blockade was found in patients with non-diabetic renal disease consuming less sodium.[24] Salt restriction is not presently recommended for patients with diabetes.

Interpretation

Our data show that modest sodium restriction added to maximal ACE inhibition reduces albuminuria by 42%. Thus, sodium restriction to 5–6 g per day should be advocated in all patients with type 2 diabetes with persisting albuminuria despite maximal ACE inhibition. Hydrochlorothiazide can be considered as a secondary treatment option when sodium restriction fails because of incompliance to a low-sodium diet, or as add-on treatment when residual albuminuria persists despite optimal sodium restriction in type 2 diabetic nephropathy. Sodium restriction in type 2 diabetic nephropathy might result in long-term improved cardiovascular and renal protection, although this remains to be shown.

sodium restriction—as noted in animal models of diabetes and in uncomplicated type 1 diabetes,[30,31] might contribute to reluctance to prescribe sodium restriction in patients with diabetes. However, our data suggest that this might not be relevant in patients with diabetic nephropathy on RAAS blockade, at least not for the extent of sodium restriction achieved here.

We acknowledge possible weaknesses in our trial. The main limitation is that we investigated short-term effects of sodium restriction and hydrochlorothiazide on intermediate endpoints only, and have no data on long-term hard endpoints and maintenance of observed effects. We included diabetic patients with albuminuria who were diagnosed as having diabetic nephropathy on the basis of medical history and analysis of blood and urine by their own nephrologists. Since renal biopsy was not required, we cannot fully exclude misclassification. Our trial includes only white patients with stable, well-preserved renal function, non-nephrotic range albuminuria, and blood pressure that was not excessively high. Whether safety or efficacy of sodium restriction and diuretics can be extrapolated to patients with worse renal function or albuminuria, severe uncontrolled hypertension, or nephrotic range proteinuria is unknown. Furthermore, because of the low number of women in our trial, the generalisability of our data to women is limited. The absence of washout periods could be deemed a limitation of our trial design, but the randomisation of treatment sequence, and the duration of the treatment periods, minimise the likelihood that carry-over effects affected our results. A limitation to the use of sodium restriction or diuretic per se is the occurrence of orthostatic complaints, as occurred quite often in this study. It is logical to assume that reduction of concomitant antihypertensive drugs could reduce the occurrence of this adverse effect, an issue to be addressed in future studies.

In conclusion, sodium restriction is an effective treatment to increase efficacy of RAAS blockade in patients with type 2 diabetic nephropathy. Sodium management is currently not included as a recommendation in the recent Kidney Disease: Improving Global Outcomes (KDIGO) guidelines[17] for diabetic nephropathy. Our data support inclusion of at least a recommendation to avoid sodium excess in patients with type 2 diabetes and nephropathy. Furthermore, in patients who do not respond adequately to sodium restriction, supplementation with diuretics should be considered.

Contributors

AJK did the trial, analysed the data, and wrote the manuscript with the assistance of SHB. JAK and FWV developed the trial protocol. MHH, A-JW, and GDL functioned as local supervisors in this multicentre trial. HG reviewed statistical analysis and manuscript. GN revised the final version of the manuscript. All authors provided substantial intellectual contribution to the manuscript, and all authors provided permission for publication.

Holland Nephrology Study (HONEST) Group

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Declaration of interests

We declare that we have no competing interests.

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