Effects of Dietary Sodium and Hydrochlorothiazide on the Antiproteinuric Efficacy of Losartan

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ABSTRACT

There is large interindividual variability in the antiproteinuric response to blockade of the renin-angiotensin-aldosterone system (RAAS). A low-sodium diet or addition of diuretics enhances the effects of RAAS blockade on proteinuria and BP, but the efficacy of the combination of these interventions is unknown. Therefore, this randomized, double-blind, placebo-controlled trial to determine the separate and combined effects of a low-sodium diet and hydrochlorothiazide (HCT) on proteinuria and BP was performed. In 34 proteinuric patients without diabetes, mean baseline proteinuria was 3.8 g/d, and this was reduced by 22% by a low-sodium diet alone. Losartan monotherapy reduced proteinuria by 30%, and the addition of a low-sodium diet led to a total reduction by 55% and the addition of HCT to 56%. The combined addition of HCT and a low-sodium diet reduced proteinuria by 70% from baseline (all $P < 0.05$). Reductions in mean arterial pressure showed a similar pattern (all $P < 0.05$). In addition, individuals who did not demonstrate an antiproteinuric response to losartan monotherapy did respond when a low-sodium diet or a diuretic was added. In conclusion, a low-sodium diet and HCT are equally efficacious in reducing proteinuria and BP when added to a regimen containing losartan and especially seem to benefit individuals who are resistant to RAAS blockade. Combining these interventions in sodium status is an effective method to maximize the antiproteinuric efficacy of RAAS blockade.

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Proteinuria is, next to high BP, a major risk factor for progression to ESRD in diabetic and nondiabetic nephropathies. Blockade of the renin-angiotensin-aldosterone system (RAAS) with angiotensin-converting enzyme inhibitors (ACEi) or angiotensin II type 1 receptor (AT1) antagonists protects against progressive renal function loss by reduction of BP and proteinuria.[1–3] However, individual differences in antiproteinuric response to RAAS blockade are large, ranging from zero to complete reduction in proteinuria.[4] It is interesting that residual proteinuria during RAAS blockade predicts the long-term renal risk for that individual patient.[5,6] Therefore, enhancing the antiproteinuric efficacy is advocated to improve renoprotection.[7]

Sodium restriction and diuretic treatment enhance the responses of proteinuria and BP to RAAS blockade.[8–10] Of note, intervention in sodium status might increase the top of the dosage-response to RAAS blockade.[9,11] Therefore, a larger maximum response can be obtained. In a previous study, we showed that the antiproteinuric response to ACEi was almost completely annihilated by high sodium intake with a blunted BP response as well.[4,9] Antiproteinuric and BP responses could be restored by addition of sodium

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restriction[9] or a diuretic.[4] Presumably, diuretics and sodium restriction enhance the efficacy of RAAS blockade by similar mechanisms—that is, by their effect on volume status. Whether their combination has added effects on the antiproteinuric efficacy of RAAS blockade is unknown. It is also unknown whether patients who are resistant to RAAS blockade have specific benefits of sodium depletion. We therefore examined the effects of sodium restriction, hydrochlorothiazide (HCT), and their combination on proteinuria and BP during losartan in proteinuric patients without diabetes in a randomized, double-blind, placebo-controlled, crossover study.

RESULTS

Patient Characteristics and Dietary Compliance

We included 34 patients (25 men, 9 women, all Caucasians) with a mean age of 50 yr (range 23–68 yr) and mean (SE) body mass index of 27.5 (0.8) kg/m^2. Baseline proteinuria was 3.8 (0.4) g/d. Diagnoses were membranous glomerulopathy (7), FSGS (7), membranoproliferative glomerulonephritis (2), minimal-change disease with secondary glomerulosclerosis (2), hypertensive nephropathy (5), IgA nephropathy (5), Alport syndrome (1), and nonconclusive diagnosis (4). One patient could not fulfill the complete protocol (because of psychological distress unrelated to the study medication) and was excluded for further analysis.

During high sodium (HS), mean urinary sodium excretion was 196 (9) mmol/d and during low sodium (LS) was 92 (8) mmol/d ($P < 0.001$), indicating an adequate dietary compliance with achieved urinary sodium values in the physiologic range (Table 1). LS was accompanied by a lower body weight in all three periods. During losartan, LS reduced body weight significantly more than HCT (Table 1). Urinary urea excretion was significantly lower during LS (Table 1).

Proteinuria and BP

Proteinuria showed a stepwise decrease. Baseline proteinuria (3.8 [0.4] g/d on placebo-HS) was significantly reduced by all interventions ($P < 0.01$ for trend; Figure 1A). Percentage change of proteinuria from baseline showed the same pattern (*i.e.*, placebo on LS induced a reduction of 22% [6%], losartan-HS 30% [4%], losartan-LS 55% [4%], losartan+HCT-HS 56% [4%], and losartan+HCT-LS 70% [4%]). The shift from HS to LS significantly reduced proteinuria during all regimens. Proteinuria was similarly reduced by addition of HCT or LS to losartan, but the lowest proteinuria was achieved with both measures combined (Figure 1A). To account for the confounding effect of he-

Table 1. Compliance to diet and effects on body weight, fractional protein excretion, protein/creatinine ratio, BP, and creatinine clearance during treatment with placebo, losartan, and losartan + HCT combined with HS and LS

Parameter (n = 33)	Placebo	Losartan	Losartan + HCT
Urinary sodium excretion (mmol/d)			
HS	200 (10)	197 (11)	193 (11)
LS	90 (10)[a]	92 (8)[a]	93 (8)[a]
Urinary urea excretion (mmol/d)			
HS	391 (16)	428 (22)[b]	436 (24)[b]
LS	338 (23)[a]	372 (27)[a,c]	362 (20)[a]
Body weight (kg)			
HS	91 (3)	90 (3)	89 (3)[b,d]
LS	89 (3)[a]	88 (3)[a,b,c]	88 (3)[a,b]
Protein excretion (ng)/ml filtrate			
HS	591 (78)[e]	387 (53)	253 (43)[d]
LS	518 (85)	286 (47)[a,b]	189 (30)[a,b,d]
Protein-creatinine ratio (mg/mg)			
HS	2.45 (0.27)[e]	1.69 (0.22)[b]	1.01 (0.15)[b,d]
LS	2.10 (0.36)	1.18 (0.19)[a,b]	0.73 (0.12)[a,b,d]
SBP (mmHg)			
HS	143 (4)[e]	135 (3)	125 (3)[b,d]
LS	137 (3)	128 (3)[a,b]	121 (2)[a,b,d]
DBP (mmHg)			
HS	86 (2)[e]	80 (2)	75 (1)[b,d]
LS	83 (1)	78 (1)[b]	74 (1)[b,d]
Creatinine clearance (ml/min)			
HS	89 (5)	94 (6)	86 (6)[d]
LS	82 (6)[a]	83 (7)[a]	75 (5)[a,b,d]

[a] $P < 0.05$ *versus* same treatment on HS (effect of LS).
[b] $P < 0.05$ *versus* placebo on same diet.
[c] $P < 0.05$ *versus* losartan + HCT HS (comparison between addition of LS and HCT with losartan).
[d] $P < 0.05$ *versus* losartan treatment on same diet (effect of HCT).
[e] $P < 0.05$ *versus* all periods.

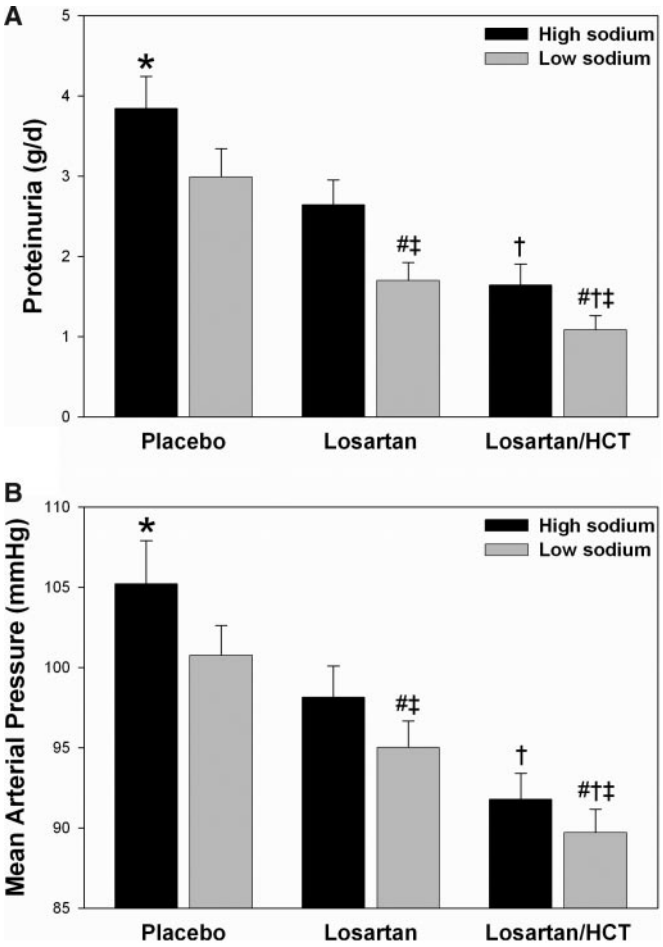

Figure 1. Intensified intervention in sodium status by combining LS and HCT is an effective tool to maximize the antiproteinuric efficacy of RAAS blockade. Proteinuria (A) and MAP (B) compared with baseline (placebo-HS) after 6-wk treatment with losartan and losartan+HCT combined with HS (■) and LS (▨). Data are means (SE). Drug effects in all periods were evaluated by a linear mixed-effect model. Tukey tests were used to localize the differences. $^{*}P < 0.05$ *versus* all periods; $^{\#}P < 0.05$ *versus* same treatment on HS (effect of LS); $^{\dagger}P < 0.05$ *versus* losartan treatment on same diet (effect of HCT); $^{\ddagger}P < 0.05$ *versus* placebo on same diet.

modynamic changes in creatinine clearance associated with BP reduction and/or RAAS blockade, we calculated protein excretion per milliliter of filtrate. This showed similar results (Table 1). The number of patients who reached target proteinuria <1 g/d was stepwise increased by intensifying antiproteinuric treatment. Target proteinuria was obtained in 6% of patients (2 of 33) by LS, in 12% (4 of 33) by losartan-HS, in 30% (10 of 33) by losartan-LS, in 33% (11 of 33) by losartan+HCT-HS, and in 49% (16 of 33) by losartan+HCT-LS. Normalization for 24-h urinary creatinine excretion did not influence the results on proteinuria (protein-creatinine ratios are shown in Table 1), indicating that the results were not influenced by urine collection errors. The differences in urinary urea excretion did not influence the results on proteinuria when urinary urea excretion was entered as covariate in the linear mixed-effect model. Therapy was equally effective in patients with primary glomerulopathies ($n = 19$) as in patients with secondary glomerulopathies (IgA or hypertensive nephropathies; $n = 10$), excluding the patients with nonconclusive diagnoses ($n = 4$; Figure 2).

BP also displayed a stepwise decrease, as shown for mean arterial pressure (MAP) in Figure 1B ($P < 0.01$ for the trend). Baseline BP (143 [4]/86 [2] mmHg on placebo-HS) was significantly reduced by all interventions (systolic [SBP] and diastolic BP [DBP] are given in Table 1). BP was similarly reduced by addition of HCT or LS during losartan, but the lowest BP was obtained by their combination. The shift from HS to LS significantly reduced MAP during all regimens.

To analyze for possible BP dependence of the antiproteinuric response, we entered BP (MAP, SBP, or DBP, respectively) as covariate in our linear effect model; however, no measure of BP contributed significantly to the model. Thus, the overall effects of therapy on proteinuria cannot be explained by the effects on BP. Moreover, baseline proteinuria but not baseline degree of BP predicted outcome in terms of residual proteinuria, with a higher residual proteinuria in patients with a higher baseline proteinuria.

Comparison of Concordance of BP and Proteinuria Responses to LS and HCT When Added to Losartan

To assess whether there are differences in mechanisms of antiproteinuric action between the two different measures of volume depletion, we analyzed the possible concordance of BP and proteinuria responses also separately for the periods with addition of LS or HCT to losartan. There was no concordance between the change in proteinuria and the change in BP when

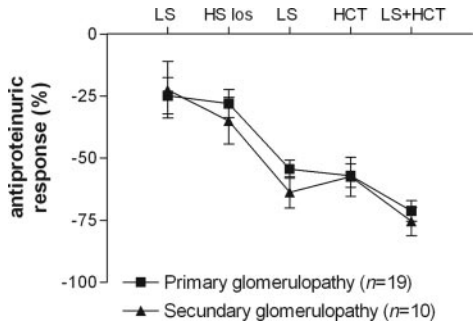

Figure 2. Conservative therapy is equally effective in patients with primary glomerulopathies as in patients with secondary nephropathies. Patients with primary glomerulopathies (membranous glomerulopathy [$n = 7$], FSGS [$n = 7$], membranoproliferative glomerulonephritis [$n = 2$], minimal-change disease with secondary glomerulosclerosis [$n = 2$], and Alport syndrome [$n = 1$]) were compared with patients with secondary renal damage (hypertensive nephropathy [$n = 5$] and IgA nephropathy [$n = 5$]), excluding the patients with nonconclusive diagnoses ($n = 4$). It is shown that conservative therapy was equally effective in the patients with primary glomerulopathies ($n = 19$) as in the patients with IgA or hypertensive nephropathies ($n = 10$). los, losartan.

patients were switched from HS to LS during losartan; however, the antiproteinuric response to HCT during losartan correlated significantly with BP effects ($R = 0.382$, $P < 0.05$; Figure 3). Furthermore, in all but one patient with a decrease in proteinuria by the addition of HCT to losartan, BP also decreased. In contrast, when LS was added to losartan, in 9 of 29 patients in whom proteinuria decreased by the addition of LS, there was no decrease in BP (Figure 3).

Renal Function

Serum creatinine was not affected by losartan during HS or by LS only. Addition of HCT or of LS to losartan significantly increased creatinine, with a further rise of borderline significance when LS was combined with HCT (Table 2). Serum urea levels were not affected by LS as such. Losartan increased urea, with a further increase when HCT was added (Table 2). HCT increased creatinine and urea levels significantly more than LS during losartan (Table 2). Creatinine clearance was lower during all sodium-restricted periods, compared with the corresponding HS periods. Losartan did not affect creatinine clearance during either diet. HCT significantly reduced creatinine clearance during losartan (Table 1).

Serum Potassium, Lipids, Albumin, Total Protein, and Uric Acid

No patients experienced hyperkalemia (>5.5 mmol/L), although serum potassium significantly increased after losartan on LS, an effect that disappeared when HCT was added. Also during HS, the addition of HCT to losartan reduced serum potassium (Table 2).

The decrease in proteinuria by LS only was accompanied by lower cholesterol levels. The antiproteinuric effect of losartan during HS and LS was also accompanied by a significant decrease in cholesterol but did not decrease further when HCT was added (Table 2). Triglycerides were unaffected by the various treatments (data not shown).

Serum total protein and albumin levels were not altered by losartan during HS, or by LS as such, but significantly increased when LS, HCT, or their combination were combined with losartan (Table 2).

The addition of HCT to losartan significantly increased uric acid during HS and LS when compared with placebo and losartan. Addition of both HCT and LS increased uric acid levels more than the addition of HCT only (Table 2).

Aldosterone and Renin Levels

Aldosterone and renin significantly increased by the shift from HS to LS during all regimens. Renin but not aldosterone was increased by losartan. HCT significantly increased aldosterone and renin during losartan. Aldosterone and renin were highest during losartan+HCT on LS (Table 2).

The effects of LS and HCT during losartan on aldosterone and renin levels were dissimilar. Addition of LS to losartan significantly increased aldosterone levels more than addition of HCT, whereas HCT increased renin levels more. Accordingly, the aldosterone-renin ratio was not affected by LS during losartan but significantly decreased when HCT was added, suggesting different effects of sodium restriction and HCT on RAAS activity and volume status. During losartan+HCT on LS, the aldosterone-renin ratio was lowest compared with all other treatment periods.

Individual Responses to Interventions

For examination of whether patients who are resistant to RAAS blockade have any specific benefits of sodium depletion, pa-

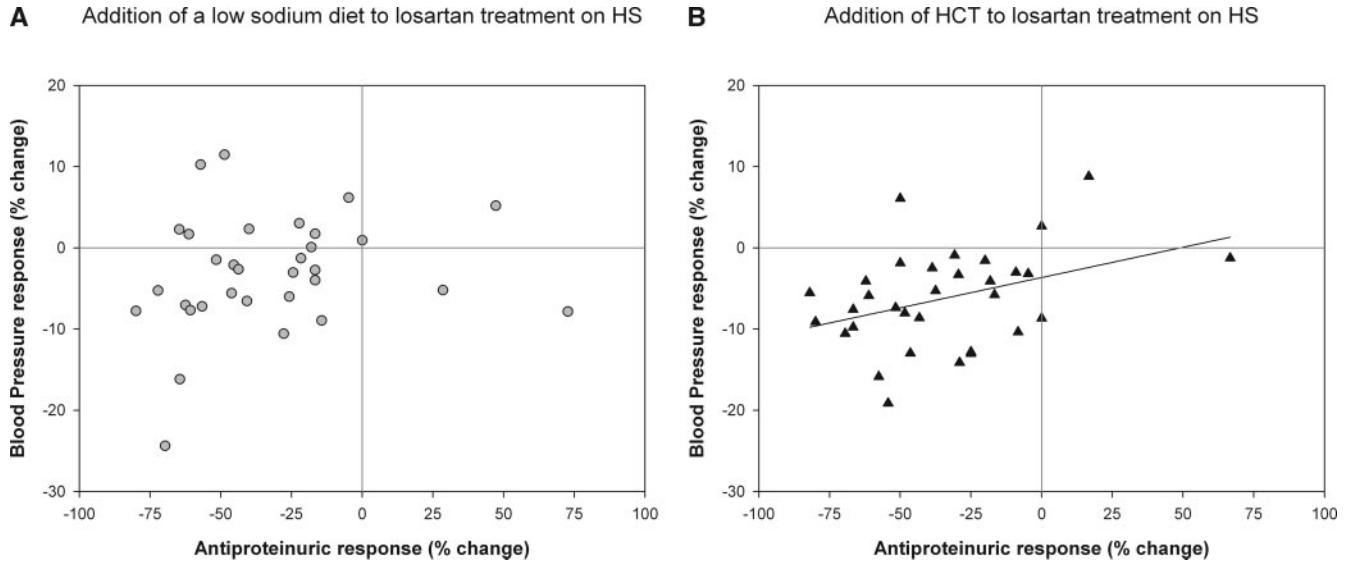

Figure 3. The antiproteinuric response of LS during losartan is not dependent on BP, in contrast to the antiproteinuric response of HCT during losartan, which correlates significantly with BP. There was no concordance between the change in proteinuria and the change in MAP when patients were switched from HS to LS during losartan (A), whereas the antiproteinuric response of HCT during losartan correlated significantly with the effects on BP ($R = 0.382$, $P < 0.05$; B).

Table 2. Effects of treatment with placebo, losartan, and losartan + HCT during HS and LS

Parameter (n = 33)	Placebo	Losartan	Losartan + HCT
Serum creatinine (μ mol/L)			
HS	125 (8)	121 (7)	136 (9)[a,b]
LS	126 (7)	129 (7)[c,d]	143 (10)[a,b]
Serum urea (mmol/L)			
HS	7.0 (0.4)	7.6 (0.5)	9.9 (0.9)[a,b]
LS	6.9 (0.5)	7.6 (0.6)[b,d]	10.8 (1.1)[a,b]
Serum potassium (mmol/L)			
HS	4.3 (0.1)	4.4 (0.1)	4.1 (0.1)[a,b]
LS	4.3 (0.1)	4.5 (0.1)[b,d]	4.0 (0.1)[a,b]
Serum total cholesterol (mmol/L)			
HS	6.1 (0.3)[e]	5.7 (0.2)	5.8 (0.2)
LS	5.9 (0.2)	5.5 (0.2)[b,d]	5.6 (0.3)[b]
Serum total protein (g/L)			
HS	68.9 (1.4)	68.0 (1.3)	69.8 (1.2)[a,b]
LS	69.2 (1.4)	70.1 (1.1)[c]	72.3 (1.1)[a,b,c]
Serum albumin (g/L)			
HS	38.7 (0.7)	38.8 (0.6)	39.7 (0.6)[a]
LS	39.0 (0.7)	40.1 (0.6)[b,c]	41.0 (0.5)[a,b,c]
Serum uric acid (mmol/L)			
HS	0.42 (0.01)	0.39 (0.01)	0.46 (0.02)[a,b]
LS	0.43 (0.02)	0.42 (0.02)[d]	0.52 (0.02)[a,b,c]
Plasma aldosterone (pg/ml)			
HS	93 (15)	78 (12)	113 (12)[a]
LS	140 (17)[c]	132 (13)[c,d]	189 (18)[a,b,c]
Plasma renin (ng AI/ml per h)			
HS	4.2 (0.4)[e]	11.1 (2.1)[b]	20.1 (2.8)[a,b]
LS	5.2 (0.5)	16.6 (2.5)[b,c,d]	37.2 (4.4)[a,b,c]
Aldosterone-renin ratio (pg/ng AI $\times$ h)			
HS	29 (5)	15 (5)[b]	10 (2)[a,b]
LS	36 (7)	16 (5)[b,d]	7 (1)[a,b,c]

[a] $P < 0.05$ *versus* losartan treatment on same diet (effect of HCT).
[b] $P < 0.05$ *versus* placebo on same diet.
[c] $P < 0.05$ *versus* same treatment on HS (effect of LS).
[d] $P < 0.05$ *versus* losartan + HCT HS (comparison between addition of LS and HCT with losartan).
[e] $P < 0.05$ *versus* all periods.

tients were divided into three groups: Resistant (<25% reduction in proteinuria, $-10 \pm 4\%$; $n = 16$), intermediate (25 to 50% reduction, $-41 \pm 3\%$; $n = 10$), and good responders (>50% reduction, $-62 \pm 3\%$; $n = 7$) according to their antiproteinuric response to losartan from baseline (on placebo-HS). Baseline characteristics (age, gender, diagnoses, body mass index, creatinine clearance), dietary compliance, and BP response to losartan did not differ between the groups (Table 3). Baseline proteinuria was similar (Figure 4, top). Antiproteinuric response in resistant and intermediate responders was significantly enhanced by LS or HCT, whereas in good responders, antiproteinuric response did not significantly improve further by LS or HCT (Figure 4, bottom). In all groups, lowest proteinuria was obtained by addition of both LS and

Table 3. Baseline characteristics according to the antiproteinuric response to losartan from baseline[a]

Parameter (n = 33)	Resistant (n = 16)	Intermediate (n = 10)	Good (n = 7)
Male/female (n)	11/5	8/2	5/2
Age (yr)	54 (3)	47 (3)	47 (6)
BMI (kg/m^2)	28 (1)	28 (2)	25 (1)
Creatinine clearance (baseline; ml/min)	88 (7)	93 (8)	85 (14)
Mean urinary sodium excretion on HS (mmol/d)	200 (11)	202 (20)	177 (10)
Mean urinary sodium excretion on LS (mmol/d)	88 (7)	93 (8)	85 (14)
BP response to losartan on HS (%)	6.2 (2.3)	4.9 (1.9)	8.1 (2.6)
Patients on additional antihypertensive drugs (n)	4/16	2/10	1/7

[a]For examination of whether patients who are resistant to RAAS blockade have any specific benefits of sodium depletion, patients were divided into three groups: resistant (<25% reduction in proteinuria; $n = 16$), intermediate (25 to 50% reduction; $n = 10$), and good responders (>50% reduction; $n = 7$) according to their antiproteinuric response to losartan from baseline (on placebo-HS). BMI, body mass index.

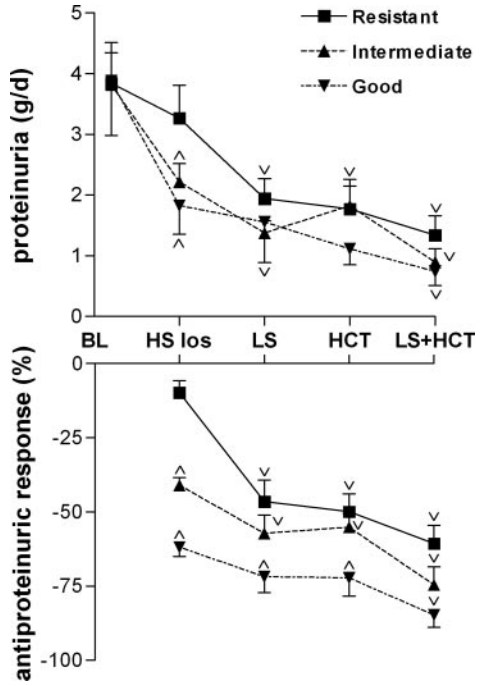

Figure 4. In proteinuric patients without diabetes, sodium depletion by LS or a diuretic is specifically beneficial in those who are resistant to RAAS blockade. We examined the effects of LS, HCT, and their combination (LS+HCT) on proteinuria (top) and antiproteinuric response (bottom) during losartan treatment (los). According to their antiproteinuric response to losartan from baseline (BL; on placebo-HS), patients were divided into three groups: Resistant (<25% reduction in proteinuria; $n = 16$), intermediate (25 to 50% reduction; $n = 10$), and good responders (>50% reduction; $n = 7$). Data are means ± SEM. $\wedge P < 0.05$ *versus* resistant patients on same treatment; $\vee P < 0.05$ *versus* losartan on HS.

HCT to losartan without differences between the groups (Figure 4, top). With this regimen, only one patient remained resistant, five were intermediate, and the number of good responders increased to 26 (χ^2: $P < 0.001$).

DISCUSSION

The objective of this study was to characterize the independent and combined effects of sodium restriction and a diuretic on the responses of proteinuria and BP to AT1-antagonist therapy in nondiabetic proteinuria. First, we found that sodium restriction and HCT were equally effective in reducing proteinuria and BP during losartan and that the largest decrease in proteinuria and BP was obtained by their combination. Second, sodium depletion by a low-sodium diet or a diuretic is specifically beneficial in patients in whom proteinuria is resistant to RAAS blockade. Third, remarkably, sodium restriction as such already exerted a modest but significant antiproteinuric effect. Our data indicate that for optimal reduction of proteinuria and BP by AT1 antagonist, both diuretic and sodium restriction should be applied.

It would be of interest to know whether the added antiproteinuric efficacy of volume depletion was due to lower BP. However, on analysis by the linear mixed-effect model, the added effects on proteinuria could not be explained by the effects on BP. Yet on univariate analysis for the different treatment periods, during HCT the effects on BP correlated to the proteinuria effects. This suggests that other antihypertensives may have had a similar effect, but we have no data to support this assumption.

The reduction of proteinuria by low sodium alone was not observed previously in patients with overt proteinuria as a result of primary glomerular disorders. It is in line with previous data on the effects of low sodium in hypertensive black patients, for whom it was attributed to the concomitant reduction in BP.[12] Moreover, recent data from our own group in healthy young adults demonstrated that sodium restriction significantly reduced urinary albumin excretion within the normal range, with only a borderline effect on BP, suggesting that direct renal effects are not excluded.[13] Obviously, the effect of sodium restriction alone in our overtly proteinuric patients was not sufficient to refrain from pharmacologic intervention. We did not study the effects of HCT monotherapy, but, in previous studies, HCT alone did not influence proteinuria.[14,15] However, reliable data on this topic are sparse and the aforementioned studies were not corrected for urinary sodium excretion or BP.[14,15]

We compared the added effects of low sodium and HCT with losartan for several reasons. First, compliance with sodium restriction can be cumbersome, and it would be convenient if there were an alternative with similar efficacy. Second, there is some evidence that the effects of sodium restriction and HCT might not be equivalent, despite that both act on sodium status. A previous study of patients with severe renal failure demonstrated that thiazides exert their antihypertensive effect by specific vascular changes, rather than by volume depletion.[16] Moreover, in uninephrectomized SHR rats, a model for hypertension and proteinuria, sodium restriction but not diuretic therapy diminished renal hypertrophy, whereas BP was similar.[17] In our study, addition of sodium restriction or HCT to losartan was equally effective in reducing proteinuria. On univariate analysis, the added effect of HCT on proteinuria was associated with BP, whereas this was not the case for the added effect of low sodium, suggesting possible different modes of action. Our data do not allow us to substantiate such mechanisms, and this issue requires further study.

Sodium restriction and HCT induced similar decreases in creatinine clearance during losartan, with the largest decrease during their combination, accompanied by a rise in serum urea. This decrease in GFR did not account for the decrease in proteinuria, as shown by the decrease in protein excretion per milliliter of filtrate, indicating a specific antiproteinuric effect of losartan beyond its effects on renal function. It is well established that during RAAS blockade, shifts in volume status exert

distinct effects on renal function, facilitating prerenal failure if volume depletion is severe. Here, the effect on renal function was mild, which is in line with relatively mild interventions in volume status. The clinical significance of the decrease in renal function for long-term outcome cannot be derived from our data, but a decrease in GFR at the onset of treatment generally predicts a subsequent slower decline of renal function, presumably because it reflects a decrease in glomerular pressure.[5,18]

Optimization of antiproteinuric treatment strategies can conceivably be obtained by combining diuretic with sodium restriction on top of RAAS blockade, although this approach might be limited by adverse events.[19] Addition of HCT to losartan significantly increased uric acid, renin, and aldosterone. Recent data showed that uric acid is an independent risk factor for the development of ESRD,[20] and experimental studies provide evidence that uric acid may be a mediator of renal disease progression.[21] Moreover, through their profibrotic actions, aldosterone[22–25] and (pro)renin[26–28] can also directly contribute to renal damage. Furthermore, a large meta-analysis in proteinuric patients without diabetes showed that the risk for ESRD was adversely influenced when proteinuria reduction by RAAS blockade was accompanied by pronounced BP reduction.[29] This suggests a J curve for BP and long-term renal outcome, with a worse outcome in patients with pronounced BP reduction. However, no adjustment for orthostasis and heart rate was made. Also, patients with lower BP might be those with more severe proteinuria and hypoalbuminemia secondary to the nephrotic syndrome, which could explain their faster progression. The assumption of a J curve for renoprotection is supported by animal studies.[30] However, for proteinuria, there is no evidence of a J curve, and the target should be <1 g/d.[31]

Several measures can maximize the effects of RAAS blockade. These include dual blockade[32] and intervention in volume status, with sodium restriction, thiazides, loop diuretics, and aldosterone blockade. Esnault *et al.*[8] showed in patients with a proteinuria comparable to ours but with a worse renal function that intensified furosemide therapy and avoiding excessive sodium on top of dual RAAS blockade resulted in a further decrease in proteinuria at the expense of a rise in serum creatinine. Our data are in line with theirs and, moreover, show the separate effects of sodium restriction and a diuretic on top of RAAS blockade by AT1 receptor blockade only. Our data suggest that further reduction of proteinuria could have been obtained by further sodium depletion, possibly at the expense of a further rise in serum creatinine. The effects of more vigorous interventions in volume status on top of dual RAAS blockade deserve further investigation. This also applies to volume intervention by aldosterone blockade that may exert specific direct renoprotective effects.[23,24,33,34] We consider it of particular interest that the sodium-depleting measures were effective in improving therapy response in patients with a poor response to monotherapy losartan.

We acknowledge possible weaknesses in our study. Urinary urea excretion was lower during sodium restriction. This could question the specificity of the effect of low sodium, because part of the antiproteinuric effect could be due to lower protein intake. We were unable to detect statistically significant effects of the differences in urinary urea excretion on proteinuria on multivariate analysis, but this does not fully exclude an effect. In a clinical outpatient setting, it is difficult to establish sodium restriction without any effect on protein intake, and *vice versa*. This was recently demonstrated in patients with stage 4/5 kidney disease in whom BP reduction by a very-low-protein diet was independently related to urinary sodium excretion but not to protein intake.[35] In an editorial commentary, it was considered likely that effects of the low-protein diet on BP were due to modification of sodium intake.[36] Here, we cannot exclude an effect of altered protein intake on proteinuria, but an effect on BP would not be likely. We studied short-term effects of sodium depletion on top of RAAS blockade on proteinuria; however, short-term reductions in proteinuria predict a slower decline in GFR in nondiabetic nephropathy.[31] Whether combined sodium depletion is effective in slowing renal function decline has to be confirmed in long-term studies.

We conclude that sodium restriction and diuretic are equally effective in reducing proteinuria and BP when added to AT1 antagonist and are specifically beneficial in patients in whom proteinuria is resistant to RAAS blockade. The largest effect on proteinuria and BP is obtained during their combination. Intensified intervention in sodium status by combining sodium restriction and diuretic is an effective tool to maximize the antiproteinuric efficacy of RAAS blockade.

CONCISE METHODS

Patients and Protocol

The protocol, which was in accordance with the Declaration of Helsinki, was approved by our local ethical committee and conducted according to the guidelines of good clinical practice. Written informed consent was obtained from each patient before inclusion. Patients were selected from our outpatient renal clinic and were enrolled between March 2004 and June 2006. All patients fulfilled the inclusion criterion of a stable proteinuria >2 g/d and <10 g/d. Only patients with stable renal function (*i.e.*, creatinine clearance >30 ml/min and <6 ml/min per yr decline) and age between 18 and 70 yr were included. Patients with uncontrolled hypertension (MAP >100 mmHg), serum potassium >5.5 mmol/L, cardiovascular disease (myocardial infarction, unstable angina, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, or stroke within the last 6 mo), contraindication for AT1-antagonist or diuretic use, and/or diabetes were excluded, as well as frequent users of nonsteroidal anti-inflammatory drugs (>2 doses/wk). Additional antihypertensive drugs except for RAAS-blocking agents or diuretics were allowed for BP control. These drugs were kept stable during the study.

Selected patients entered this single-center, prospective, randomized, placebo-controlled, crossover study and were consecutively treated during 6 wk with placebo, losartan (100 mg once daily; Cozaar [Merck & Co. Inc., Whitehouse Station, NJ]), and losartan plus HCT

(100/25 mg once daily; Fortzaar [Merck & Co. Inc.]) in random order. Patients were instructed to take the study medication once daily, in the morning, except on study days; on those days, the study drug was not taken before data collection at the hospital (between 8:00 and 9:30 a.m.), allowing BP measurement at trough. At the same time, patients were randomly assigned to either a high-sodium diet (200 mmol Na^+/d [approximately 4.8 g]) or a low-sodium diet (50 mmol Na^+/d [approximately 1.2 g]) during 18 wk (three 6-wk periods). After 18 wk, patients changed diet and the three 6-wk periods were repeated.

During the whole study, patients were closely supported by a dietary consultant and were instructed to adhere to a stable protein diet (1.1 g/kg per d) throughout the study. Differences in sodium intake were achieved by replacing sodium-rich products with a low-sodium product of the same product group to remain isocaloric with a similar balance among protein, carbohydrate, and fat. For prevention of concurrent changes in dietary habits, the diets were based on the personal food habits of each patient and fitted to the individual caloric need. Every 2 wk after an overnight fast, patients collected 24-h urine, BP was measured, and blood was sampled to control dietary compliance and to monitor renal function and BP. Collected data at the end of each 6-wk treatment period were used for analysis.

Study Measures

The primary end point was the 24-h proteinuria at the end of each treatment period. Secondary end points were the MAP and serum creatinine, urea, cholesterol, triglycerides, total protein, and albumin. At the end of each period, on the day before every visit, patients collected 24-h urine samples to determine proteinuria and urinary sodium, urea, potassium, and creatinine excretion. Urinary protein was determined using the pyrogallol red-molybdate method. Serum and urinary electrolytes and creatinine and cholesterol, triglycerides, total protein, and albumin levels were determined using an automated multi-analyzer (SMA-C; Technicon, Tarrytown, NY). Aldosterone was measured with a commercially available RIA kit (Diagnostic Products Corp., Los Angeles, CA). Plasma renin activity was measured as described previously with a RIA that detects the amount of angiotensin I produced per hour in the presence of excess angiotensinogen (nanograms of angiotensin I produced per milliliter of plasma per hour). This assay measures the enzymatic activity of active plasma renin in the presence of an excess of its (exogenous) substrate.[37] BP was measured under constant conditions, at 1-min intervals by an automatic device (Dinamap; GE Medical systems, Milwaukee, WI), with the patient in semisupine position. After 15 min of measurements, the mean of the last four readings was used for further analysis. MAP was calculated as the sum of one third of SBP and two thirds of the DBP.

Sample Size

We hypothesized that patients would present a mean $\pm$ SD proteinuria of 3.5 $\pm$ 2 g/d at baseline (placebo on HS). Assuming a reduction of 1.0 $\pm$ 0.7 g/d with losartan and an extra reduction of 0.6 $\pm$ 0.7 g/d by the addition of HCT or LS, it was estimated that 32 patients had to complete the crossover design sequence to give the study a 90% power to detect a statistically significant difference ($\alpha = 0.05$). On the basis of an expected dropout rate of 5%, we included 34 patients.

Data Analysis

Results are expressed as mean and SE. Baseline data were obtained after 6-wk placebo treatment with HS. Drug effects in all periods were evaluated using linear mixed-effect models. Tukey tests were used to localize the differences. Statistical significance was assumed at the 5% level of probability. We used SPSS 12.0.1 for Windows (SPSS Inc., Chicago, IL) for all analyses.

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DISCLOSURES

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