Supplemental Material

A Randomized Trial of Distal Diuretics versus Dietary Sodium Restriction for Hypertension in Chronic Kidney Disease

Dominique M. Bovée, Wesley J. Visser, Igor Middel, Anneke De Mik – van Egmond,
Rick Greupink, Rosalinde Masereeuw, Frans G.M. Russel,
A.H. Jan Danser, Robert Zietse, Ewout J. Hoorn

Table of Contents:

Supplemental Methods

Urinary aldosterone measurements were performed using a commercial assay (Demeditec, Germany). The detection limit and mean coefficient of variation of the within-run and between-run variability of this assay are 1.44 pg/mL, 3.3 % and 8.4 %, respectively. Moreover, the antibody used in the immunoassay is highly specific for aldosterone. Extremely low cross-reactivities were obtained against other naturally occurring steroids (cortisone, corticosterone, DHEAs, etc.). Urinary renin and angiotensinogen measurements were performed using an in-house developed enzyme-kinetic assay. The detection limit and mean coefficient of variation of the within-run and between-run variability of this assay are 2 pg/mL, 2.9% and 12.6% for renin measurements and 0.12 ng/mL, 4% and 10% for angiotensinogen measurements. Please note that in every enzyme-kinetic assay, we measure a set of standard samples allowing us to determine the within- and between-run variability. Furthermore, we always include buffer samples to correct for background noise. As an example, when measuring renin in the presence of excess angiotensinogen, the same amount of excess angiotensinogen is incubated with buffer, and the generated amount of angiotensin I is subtracted from the amount of angiotensin I generated in the actual sample. This needs to be taken into account, particularly when measuring samples with low renin levels like urine.[1] In previous studies we have added fixed amounts of (pro)renin and angiotensinogen to urine samples, and found recovery to be >95%.[2, 3] This is not surprising, since the levels of degrading enzymes in urine are negligible. Finally, regarding cross-reactivity, it is important to note that the assays rely on the detection of angiotensin I making use of an angiotensin I-directed antibody. Cross-reactivity of this antibody with other angiotensin metabolites is <0.1%.[4] In this regard, the correction for background noise as explained above seems to be of greater importance, since the assay is performed in the presence of angiotensinase inhibitors, not allowing the generation of metabolites other than angiotensin I.

Supplemental Table 1: Consolidated Standards of Reporting Trials (CONSORT) 2010 checklist of information.

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomized trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4-5
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6-7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	6
	4b	Settings and locations where the data were collected	6
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6-7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	7-8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomization:			
Sequence generation	8a	Method used to generate the random allocation sequence	7
	8b	Type of randomization; details of any restriction (such as blocking and block size)	7
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	7
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	18
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	NA
	11b	If relevant, description of the similarity of interventions	4
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	8-9
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	8-9
Results			

Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome	10
	13b	For each group, losses and exclusions after randomization, together with reasons	10
Recruitment	14a	Dates defining the periods of recruitment and follow-up	6
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	19
Numbers analyzed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	8 & 10
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	10-12
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	10-12
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	20
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	16
Generalizability	21	Generalizability (external validity, applicability) of the trial findings	13-17
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13-17
Other information			
Registration	23	Registration number and name of trial registry	6
Protocol	24	Where the full trial protocol can be accessed, if available	NA
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	18

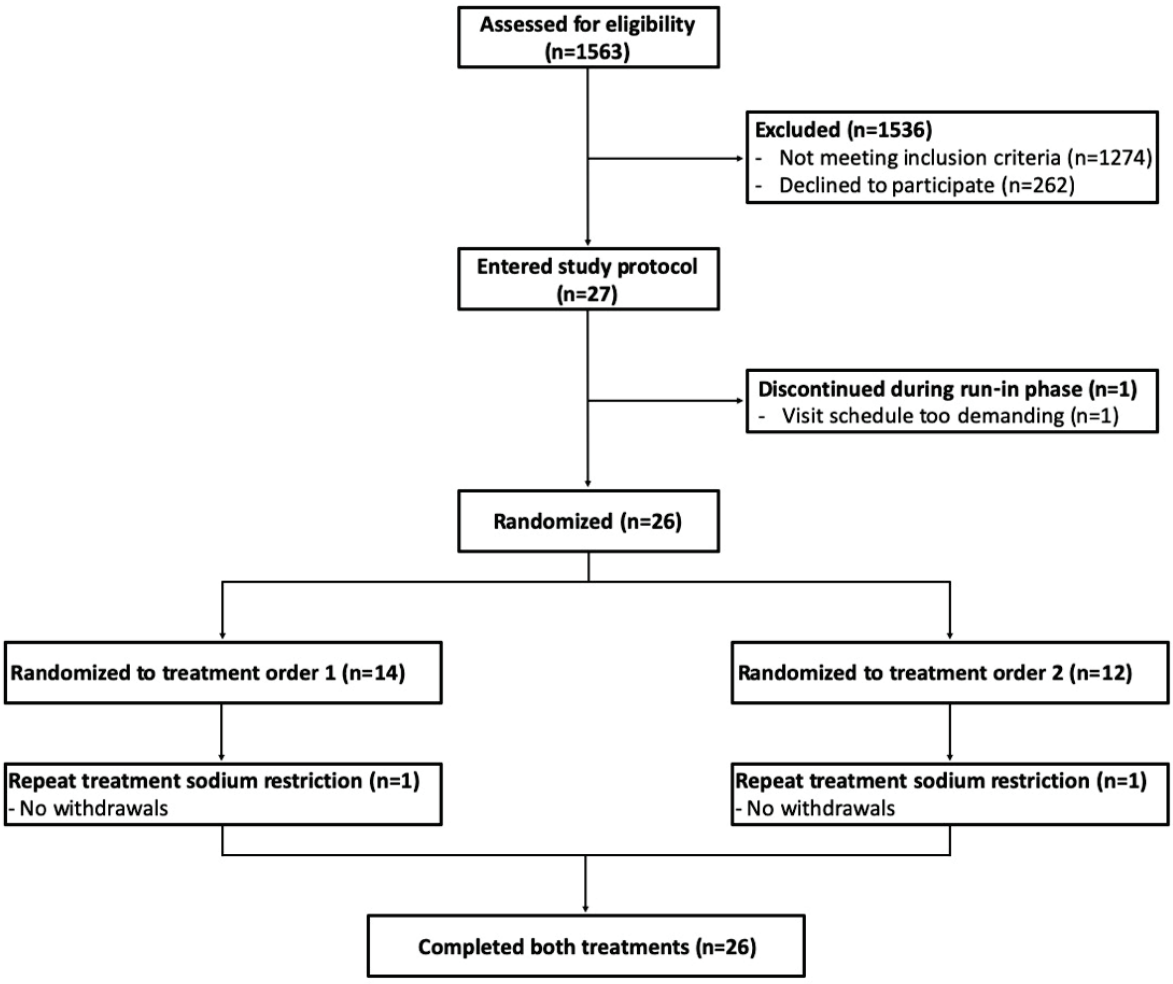
Supplemental Table 2: Effect of sodium restriction and diuretics on day and night systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP).

	Na^+ restriction	*P* for treatment effect	Distal diuretics	*P* for treatment effect	*P* for interaction
Day SBP (mmHg)	–6 ± 2	$P < 0.01$	–14 ± 2	$P < 0.01$	$P < 0.01$
Day DBP (mmHg)	–2 ± 1	$P = 0.2$	–5 ± 1	$P < 0.01$	$P = 0.09$
Day MAP (mmHg)	–4 ± 1	$P < 0.05$	–8 ± 1	$P < 0.01$	$P < 0.05$
Night SBP (mmHg)	–3 ± 2	$P = 0.4$	–14 ± 2	$P < 0.01$	$P < 0.01$
Night DBP (mmHg)	0 ± 2	$P = 1$	–5 ± 2	$P < 0.01$	$P < 0.05$
Night MAP (mmHg)	–1 ± 2	$P = 0.8$	–8 ± 2	$P < 0.01$	$P < 0.01$
SBP dipping (%)	+2.1 ± 3.4	$P = 1$	+1.4 ± 3.4	$P = 1$	$P = 1$

Treatment effect and treatment interaction were analyzed by two-way repeated measures ANOVA that included treatment order as between-subject factor.

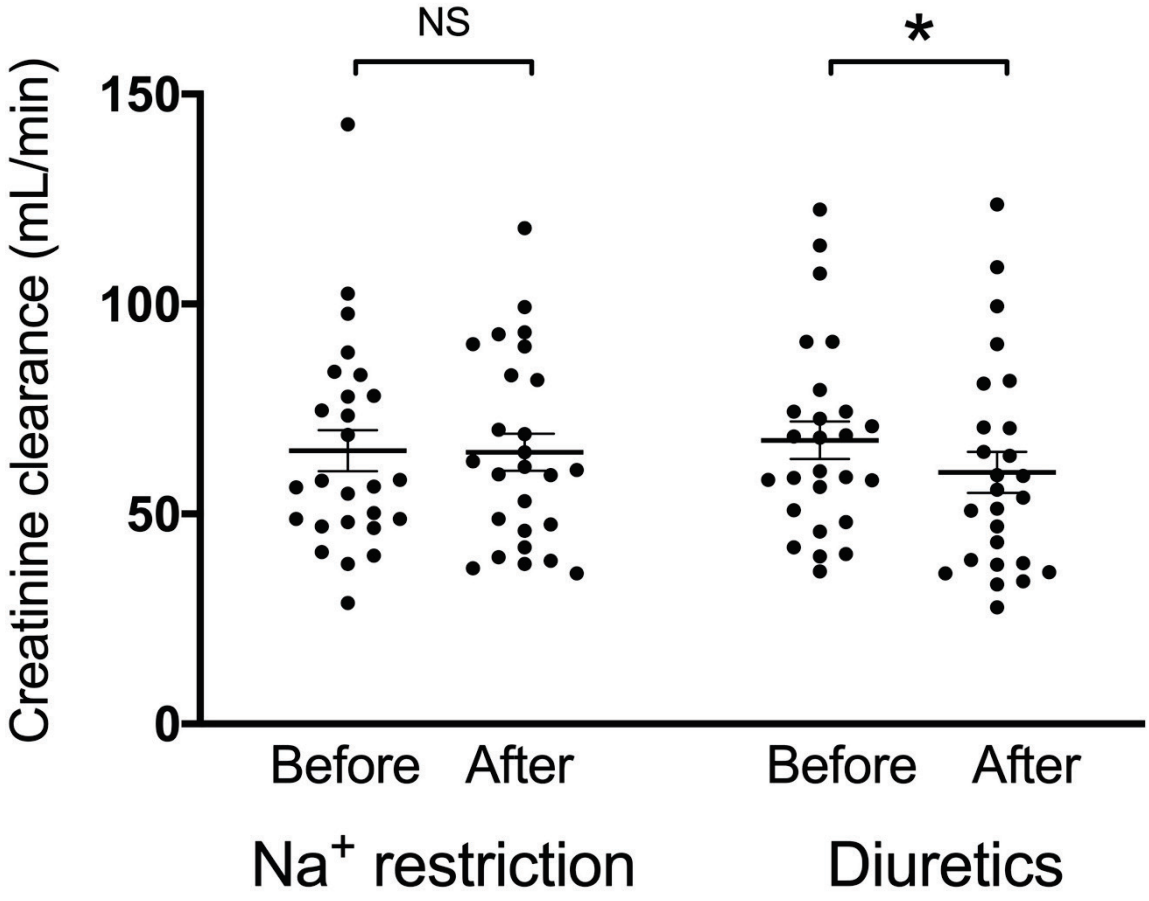
DBP, diastolic blood pressure; MAP, mean arterial pressure; Na^+, sodium; SBP, systolic blood pressure.

Supplemental Figure 1: Consolidated Standards of Reporting Trials (CONSORT) diagram.



Legend: Treatment order 1: first treatment period diuretics, second treatment period dietary sodium restriction. Treatment order 2: first treatment period sodium restriction, second treatment period diuretics.

Supplemental Figure 2: Effect of sodium (Na^+) restriction and diuretics on creatinine clearance.



Legend: Data are normally distributed and two-way repeated measures ANOVA was used for analysis. * $P < 0.05$ for difference before versus after treatment.

References

1. van Kesteren CA, Saris JJ, Dekkers DH, Lamers JM, Saxena PR, Schalekamp MA and Danser AH. Cultured neonatal rat cardiac myocytes and fibroblasts do not synthesize renin or angiotensinogen: evidence for stretch-induced cardiomyocyte hypertrophy independent of angiotensin II. *Cardiovasc Res*. 1999;43:148-56.

2. van den Heuvel M, Batenburg WW, Jainandunsing S, Garrelds IM, van Gool JM, Feelders RA, van den Meiracker AH and Danser AH. Urinary renin, but not angiotensinogen or aldosterone, reflects the renal renin-angiotensin-aldosterone system activity and the efficacy of renin-angiotensin-aldosterone system blockade in the kidney. *J Hypertens*. 2011;29:2147-55.

3. Roksnoer LC, Verdonk K, Garrelds IM, van Gool JM, Zietse R, Hoorn EJ and Danser AH. Methodologic issues in the measurement of urinary renin. *Clin J Am Soc Nephrol*. 2014;9:1163-7.

4. Danser AH, Koning MM, Admiraal PJ, Derkx FH, Verdouw PD and Schalekamp MA. Metabolism of angiotensin I by different tissues in the intact animal. *Am J Physiol*. 1992;263:H418-28.