Response to angiotensin-converting enzyme inhibition is selectively blunted by high sodium in angiotensin-converting enzyme DD genotype: evidence for gene–environment interaction in healthy volunteers

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Background **Renin–angiotensin–aldosterone system blockade is a cornerstone in cardiovascular protection. Angiotensin-converting enzyme (ACE)-DD genotype has been associated with resistance to angiotensin-converting enzyme inhibition (ACEi), but data are conflicting. As sodium intake modifies the effect of ACEi as well as the genotype–phenotype relationship, we hypothesize gene–environment interaction between sodium-status, the response to ACEi, and ACE genotype.**

Method **Thirty-five male volunteers (26 ± 9 years; II $n = 6$, ID $n = 18$, DD $n = 11$) were studied during placebo and ACEi (double blind, enalapril 20 mg/day) on low [7 days 50 mmol Na^+/day (low salt)] and high [7 days 200 mmol Na^+/day (high salt)] sodium, with a washout of 6 weeks in-between. After each period mean arterial pressure (MAP) was measured before and during graded infusion of angiotensin II (Ang II).**

Results **During high salt, ACEi reduced MAP in II and ID, but not in DD [II: 88 (78–94) versus 76 (72–88); ID: 87 (84–91) versus 83 (79–87); both $P < 0.05$ and DD: 86 (82–96) versus 88 (80–90); ns, $P < 0.05$ between genotypes]. However, during low salt, ACEi reduced MAP in all genotype groups [II: 83 (78–89) versus 77 (72–83); ID: 88 (84–91) versus 82 (78–86); DD: 84 (80–91) versus 81 (75–85); all $P < 0.05$]. During high salt + ACEi, the Ang II response was blunted in DD, with an 18% rise in MAP during the highest dose versus 22 and 31% in ID and II ($P < 0.05$). Low salt annihilated these differences.**

Conclusion **In healthy participants, the MAP response to ACEi is selectively blunted in DD genotype during high salt, accompanied by blunted sensitivity to Ang II. Low salt corrects both abnormalities. Further analysis of this gene–environment interaction in patients may contribute to strategies for improvement of individual treatment efficacy.**
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Keywords: angiotensin-converting enzyme genotype, angiotensin-converting enzyme inhibition, angiotensin II infusion, blood pressure, sodium intake

Abbreviations: ACE, angiotensin-converting enzyme; ACEi, angiotensin-converting enzyme inhibition; Ang I, angiotensin I; Ang II, Angiotensin II; MAP, mean arterial pressure; PRA, plasma renin activity; RAAS, renin–angiotensin–aldosterone system

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Introduction

The renin–angiotensin–aldosterone system (RAAS) is important in the regulation of blood pressure, volume homeostasis, and cardiovascular and renal pathophysiology. The angiotensin-converting enzyme (ACE), which converts angiotensin I (Ang I) into angiotensin II (Ang II), is a key enzyme in the RAAS. The ACE insertion/deletion (I/D) genotype is a main determinant of plasma and tissue ACE levels, which are highest in the DD genotype, lowest in II genotype and intermediate for heterozygotes [1]. By this effect on ACE activity, the ACE I/D genotype was assumed a plausible candidate gene for determining the response to angiotensin-converting enzyme inhibition (ACEi). This issue was addressed in many association studies in hypertension, renal, and cardiovascular disease [2–4]. However, results are conflicting. A possible explanation for the divergent results might be heterogeneity in other factors relevant to therapy response.

Sodium status is a well established environmental determinant of the response to ACEi [5–7]. Surprisingly, its impact on a possible association between ACE genotype and response to ACEi has not been considered. Previously, we demonstrated gene–environment interaction between sodium status and ACE I/D genotype in healthy volunteers. The responses to Ang I in DD homozygotes as compared to II and ID genotype were enhanced during high, but not during low sodium intake in the same participants [8]. Retrospective cross-sectional data in

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renal patients suggest gene–environment interaction between sodium status, ACE I/D genotype and the response to ACEi. In DD participants, a high sodium intake was associated with poor responses of blood pressure and proteinuria to ACEi, whereas in ID and II participants a relationship between sodium intake and therapy response was not readily apparent [9].

These data elicit the hypothesis that in DD genotype the response to ACEi is more strongly dependent on concomitant sodium restriction than in II and ID genotype. To prospectively test this hypothesis we studied the blood pressure response to ACEi in a placebo-controlled design and investigated the effects of a shift in sodium intake during therapy in relation to ACE I/D genotype.

ACE I/D genotype may also be involved in susceptibility to progressive renal and cardiovascular damage, although data are conflicting [10–12]. Moreover, cardiovascular and renal damage can increase tissue ACE activity [13–15]. To avoid interaction or confounding by genotype-associated differences in underlying damage, the current study was conducted in healthy volunteers. Finally, to investigate whether differences in response to ACEi might be related to differences in endogenous tissue RAAS activity, the pressor responses to Ang II were assessed during all four conditions.

Methods

Participants

Thirty-five healthy normotensive white men (age median: 24, 25–75%, 21–27 year) were recruited for the study. Normal blood pressure was defined as systolic blood pressure lower than 140 mmHg and diastolic blood pressure lower than 90 mmHg. All had medical histories without significant disease, used no medication, and physical examination results were unremarkable. Written informed consent was obtained after a full explanation of the study. The protocol was approved by the Ethics Committee of the University Medical Center of Groningen and in accord with the Declaration of Helsinki Principles.

Study design

The protocol had a double-blind placebo-controlled design, and consisted of two 2-week periods, during which the participants received either placebo or enalapril 20 mg/day capsules, separated by a 6-week washout period. Both periods were divided into a 7-day period on low-sodium diet (low salt; aim: 50 mmol Na^+/day) followed by a 7-day period on high-sodium diet (high salt; aim: 200 mmol Na^+/day). Differences in sodium intake were achieved by replacing sodium-rich products with a low-sodium product of the product group to remain isocaloric with a similar balance between protein, carbohydrate, and fat. To prevent inadvertent concurrent dietary changes, the diets were based on the individual food habits and caloric requirements. In healthy young participants, sodium restriction induces RAAS activation within 3 days, associated with reestablishment of sodium balance [16]. A week has been demonstrated to be sufficient for stabilization of circulating hormones [17]. On days 4 and 6 of each dietary period, participants collected 24-h urine to assess the achievement of a stable sodium balance. No difference in sodium excretion between day 3 and day 6 was observed, indicating a stable sodium balance during the experiment. The mean 24-h urinary excretion at day 6 is presented in Table 1. At day 6, medication was taken during the evening before midnight.

Having abstained from food, alcohol, fluids, and strenuous exercise for 12 h, participants reported to the research unit at 0800 h, on day 7. An intravenous cannula was inserted into each forearm, one for drawing blood samples, the other for infusion of Ang II. All participants received standardized meals and fluids during the day, with sodium intake adjusted to the prescribed diet. Blood pressure was measured at 15-min intervals using a non-invasive device (Dinamap; GE Medical Systems, Milwaukee, Wisconsin, USA). Mean arterial pressure (MAP) was calculated as diastolic pressure plus one-third of the pulse pressure. Baseline values for blood pressure were obtained from 1000 to 1200 h. Between 1200 and 1500 h.

Table 1 Baseline characteristics during low and high sodium diet on placebo and during angiotensin-converting enzyme inhibition

	II	ID	DD
N	6	18	11
Age (year)	22.5 (21.1–27.2)	23.2 (21.6–25.5)	26.1 (20.7–39.0)
Low sodium diet			
Body weight (kg)	76.3 (72.8–84.2)	78.8 (72.5–84.8)	77.6 (70.3–88.3)
Urinary Na^+ (mmol/day)	39 (19–77)	37 (13–53)	32 (19–54)
High sodium diet			
Body weight (kg)	78.1 (74.4–85.3)*	80.7 (73.6–85.7)*	78.7 (71.0–88.2)*
Urinary Na^+ (mmol/day)	206 (172–226)*	219 (177–252)*	234 (173–273)*
Low sodium diet + ACE inhibition			
Body weight (kg)	76.6 (72.9–83.6)	77.9 (71.9–83.2)	76.2 (70.2–86.2)
Urinary Na^+ (mmol/day)	49 (31–66)	45 (31–66)	47 (39–80)
High sodium diet + ACE inhibition			
Body weight (kg)	77.7 (73.6–85.8)*	80.6 (74.2–85.4)*	78.5 (72.8–88.1)*
Urinary Na^+ (mmol/day)	185 (148–253)*	216 (166–259)*	238 (185–268)*

Medians and interquartile ranges are given. * $P < 0.05$, compared to low sodium.

Ang II (Clinalfa, Merck Biosciences AG, Läufelfingen, Switzerland) was administered intravenously, at a constant rate in doses of 0.3 ng/kg per h; *d* at a constant rate of 1 and 3 ng/kg per h, during the subsequent 1-h periods. During the Ang II infusions blood pressure was measured at 5-min intervals.

Blood sampling and analysis

Blood samples were drawn at 0800 and at 1000 h, and hourly thereafter until 1500 h, in semisupine position, in prechilled tubes, and immediately centrifuged at 4°C. Plasma and serum for measurement of aldosterone, active plasma renin concentration (APRC) and ACE activity was stored at −20°C until analysis. Blood samples for determination of angiotensins were drawn in cold, standard 3 ml vacuum tubes containing 5.4 μg K_3EDTA and an additional 0.2 ml ACE inhibitor cocktail containing 1.704 μg phenantroline, 0.16 mg enalaprilat, 1 ml ethanol, and 4 mg neomycin. After centrifugation at 4°C, the plasma was snap-frozen and stored at −80°C until analysis. Plasma concentrations of Ang I and Ang II were measured after SepPak extraction of plasma samples and HPLC separation [18]. Radioimmunoassays of dried collected fractions were used for quantification of angiotensins using specific antibodies. Detection limits were 0.5 to 1.0 fmol/tube. Aldosterone was measured with a commercially available radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, California, USA). APRC was determined in terms of Ang I generation using a radioimmunoassay [19]. ACE activity was determined as the amount of hippuric acid produced by cleavage of the commonly used substrate hippuryl-histidyl-leucine [20].

ACE genotypes were determined by using Taqman-PCR, as described previously [21]. We determined the rs4341 single nucleotide genotype, which is in equilibrium with the ACE insertion/deletion (I/D) genotype [21]. The ABI PRISM 7000 SDS instrument in conjunction with the ABI TaqMan Universal Master Mix (Applied Biosystems, Darmstadt, Germany) was used to perform the assays. The following primers and probes were used fluorogenic probes, 5′-VIC-CTCAAGCCAT TCAA and 5′-FAM-CTCAAGGCATTCAA and primers, AGCAGAGGTGAGCTAAGGGCT and GGCCATC ACATTGTCAGATCT.

Urinary concentrations of sodium were measured by standard autoanalyzer technique (MEGA; Merck, Darmstadt, Germany). Urinary albumin excretion was determined by nephelometry with a threshold of 2.3 mg/l (Dade Behring Diagnostic, Marburg, Germany).

Data analysis

The sample size calculation is based on an extrapolation of the blood pressure response to ACEi therapy from earlier observations in renal patients, taking into account that the response in healthy participants would be diminished [9]. A sample size of 18 participants provided 80% power to detect an 8-mmHg or greater difference in MAP between II and DD genotype. We postulated that the ACE genotype would be in Hardy–Weinberg equilibrium. The ID genotype frequency was therefore expected to be twice as high as the II and DD genotype frequency, resulting in an overall sample size of 36 participants.

Baseline values are expressed as median plus interquartile range. The responses in MAP to a shift from low to high sodium, ACEi therapy and Ang II are expressed as percentage change compared with baseline with blood pressure from 1000 to midnight. (at 15-min intervals) as baseline values. The percentage change in MAP during each infusion step was analyzed as the average of the last five measured values during the hour (at 5 min intervals). Because of small sample size, nonparametric tests were used. For comparison between the different periods the paired Wilcoxon signed rank test was used. Differences between the genotypes were tested with the Kruskal–Wallis *H* test and Mann–Whitney *U* test. Correlation coefficients were analyzed using linear regression analysis. Spearman correlation coefficients are presented. A multivariate analysis was performed to calculate whether differences in baseline blood pressure were determinants of Ang II sensitivity, with the pressor response to Ang II as the dependent variable and baseline blood pressure and ACE activity as independent variables. The null hypothesis was rejected when *P* value was 0.05 or less.

Results

Clinical parameters

Data are presented by a break-up by ACE I/D genotype. Six participants had the II genotype, 18 the ID genotype, and 11 the DD genotype, thus being in Hardy–Weinberg equilibrium, with an allele frequency in line with other studies in the Netherlands.

Dietary compliance was good during all study conditions without difference between the genotypes (Table 1). As anticipated, higher sodium intake elicited a rise in body weight during both placebo and ACEi, without differences between the genotypes. Urinary albumin excretion was below the threshold in two participants; average values were 5.1 (3.4–7.1) mg/24 h, without any participant being in the microalbuminuric range.

Blood pressure

During placebo there were no differences in MAP between the genotypes on either sodium intake (Table 2). During ACEi, MAP was significantly lower than during placebo on both sodium intakes in II and ID participants (both $P < 0.05$). However, in DD participants MAP during ACEi was lower than placebo only during low sodium intake, but not during high sodium intake (DD: low salt versus low salt + ACEi, $P < 0.05$; high salt versus high salt + ACEi, ns). Consequently, only during the high

Table 2 Mean arterial blood pressure during low and high sodium diet on placebo and during angiotensin-converting enzyme inhibition at baseline and during angiotensin II infusion (0.3, 1, and 3 ng/kg per min)

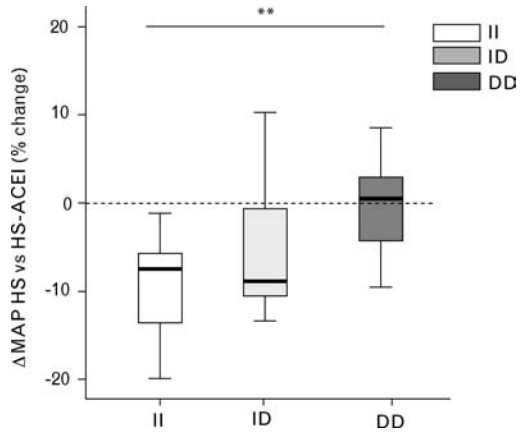
	II	ID	DD
Low sodium diet			
Baseline – MAP (mmHg)	83 (78–89)	88 (84–91)	84 (80–91)
0.3 ng/kg per min	84 (82–89)	87 (83–92)	86 (80–89)
1 ng/kg per min	92 (90–98)	90 (86–99)	93 (88–99)
3 ng/kg per min	100 (95–107)	99 (95–104)	97 (90–106)
High sodium diet			
Baseline – MAP (mmHg)	88 (78–94)	87 (84–91)	86 (82–96)
0.3 ng/kg per min	87 (83–97)	87 (83–93)	90 (84–93)
1 ng/kg per min	99 (90–103)	93 (88–99)	95 (91–102)
3 ng/kg per min	102 (98–114)	103 (96–112)	106 (97–110)
Low sodium diet + ACE inhibition			
Baseline – MAP (mmHg)	77 (72–83)*	82 (78–86)*	81 (75–85)*
0.3 ng/kg per min	79 (74–90)	83 (77–86)	80 (76–90)
1 ng/kg per min	82 (74–100)	87 (85–92)	87 (84–93)
3 ng/kg per min	90 (83–119)	93 (91–101)	97 (91–100)
High sodium diet + ACE inhibition			
Baseline – MAP (mmHg)	76 (72–88)*	83 (79–87)*	88 (80–90) ns,#,†
0.3 ng/kg per min	79 (72–98)	86 (82–88)	86 (81–98)
1 ng/kg per min	88 (84–110)	90 (87–95)	89 (84–102)
3 ng/kg per min	99 (93–119)	100 (96–105)	104 (92–111)

Medians and interquartile ranges are given. ACE, angiotensin-converting enzyme; MAP, mean arterial pressure; ns, not significant compared with high sodium * $P < 0.05$, compared with placebo on same sodium diet. # $P < 0.05$, compared with low sodium + ACEi. † $P < 0.05$, compared with II.

sodium + ACEi combination, MAP was significantly different between the genotypes, being highest in DD, intermediate in ID and lowest in II (88 versus 83 versus 76 mmHg; Kruskal–Wallis, $P < 0.05$).

Therapy response to ACEi expressed as percentage difference in MAP between placebo and ACEi during high sodium is given in Fig. 1, showing the stepwise difference in blood pressure response between the genotypes, with the largest response in II and absence of the response in DD ($P < 0.05$). The effect of the shift from low to high sodium intake during ACEi is given in Fig. 2, showing that high sodium blunted the effect of ACEi on MAP in the DD genotype only ($P < 0.05$), with a virtually negligible impact in II and ID genotype. Essentially similar results were obtained for systolic and diastolic blood pressure response (supplemental Figure 1, http://links.lww.com/HJH/A45).

Fig. 1

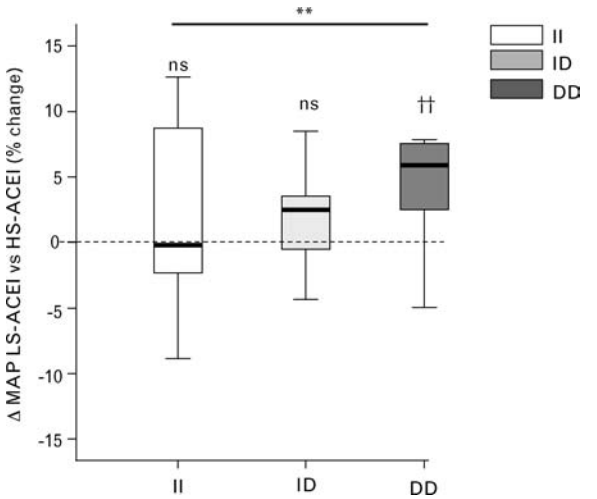


Blunted therapy response to angiotensin-converting enzyme inhibition (ACEi) in DD participants during high sodium. Median (interquartile range) difference (%) in MAP between placebo and ACEi for the different genotypes. ** $P < 0.05$ DD versus ID and II.

Ang II infusion

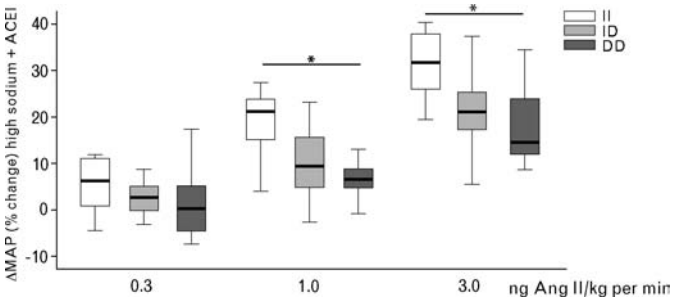
Ang II infusion induced a dose-dependent increase in blood pressure during either sodium intake on placebo and ACEi (Table 2). The lowest dose (0.3 ng/kg per min) of Ang II did not affect blood pressure, except for the ID

Fig. 2



Change in MAP (%) during ACEi therapy elicited by the shift from low to high sodium for the different genotypes. Median (interquartile range) is given. ACEi, angiotensin-converting enzyme inhibition. †† $P < 0.05$ low salt + ACEi versus high salt + ACEi in DD, not significant, low salt + ACEi versus high salt + ACEi in II and ID. ** $P < 0.05$ DD versus ID and II.

Fig. 3



Blunted reaction to angiotensin II infusion in DD genotype during high sodium + ACEi. Responses of mean arterial blood pressure to infusion of angiotensin II 0.3, 1, and 3 ng/kg per min. Mean and SEM is given, ACEi, angiotensin-converting enzyme inhibition, $^{*}P < 0.05$ II versus DD.

genotype during high sodium + ACEi. All further changes from baseline were significant (all genotypes: baseline versus 1 and 3 ng, $P < 0.05$).

The blood pressure response to Ang II infusion were significantly larger during high sodium than during low sodium, both on placebo and during ACEi, when all genotypes were analyzed together (% change in MAP during 3 ng Ang II: low salt 16 (10–21)% versus high salt 20 (13–27)% and low salt + ACEi 16 (12–23)% versus high salt + ACEi 21 (14–29)%, both $P < 0.05$). The pressor responses to Ang II infusion were similar for the genotypes during either sodium intake on placebo and during low sodium + ACEi. However, during high sodium + ACEi, the response to Ang II was lowest in the DD genotype, intermediate in ID genotype and highest in the II genotype (0.3 ng, ns; 1.0 and 3 ng, $P < 0.05$) (Fig. 3).

Circulating parameters of renin–angiotensin–aldosterone system-activity

Baseline plasma RAAS parameters are shown in Table 3. During placebo, serum ACE activity was higher among DD and ID participants compared with II during both sodium intakes (low salt and high salt: II versus DD and ID, $P < 0.05$). During low sodium + ACEi, ACE activity was higher in DD compared to II genotype (low salt + ACEi: II versus DD, $P < 0.05$). ACEi significantly reduced serum ACE activity during both sodium intakes without differences between the genotypes. As anticipated, the shift in sodium intake during both placebo and ACEi did not affect ACE activity.

During placebo, plasma renin activity (PRA), aldosterone, Ang I, and Ang II concentrations were approximately threefold lower during high sodium. ACEi elicited a significant rise in PRA and Ang I on both sodium intakes. Aldosterone was reduced by ACEi, only significantly in ID participants. Ang II levels decreased only significantly for ID and II participants during low sodium + ACEi. The shift in sodium intake during ACEi induced a significant fall in PRA, aldosterone and Ang I levels. During all four conditions the PRA, aldosterone, Ang I, and Ang II levels were similar for the genotypes.

Angiotensin-converting enzyme activity

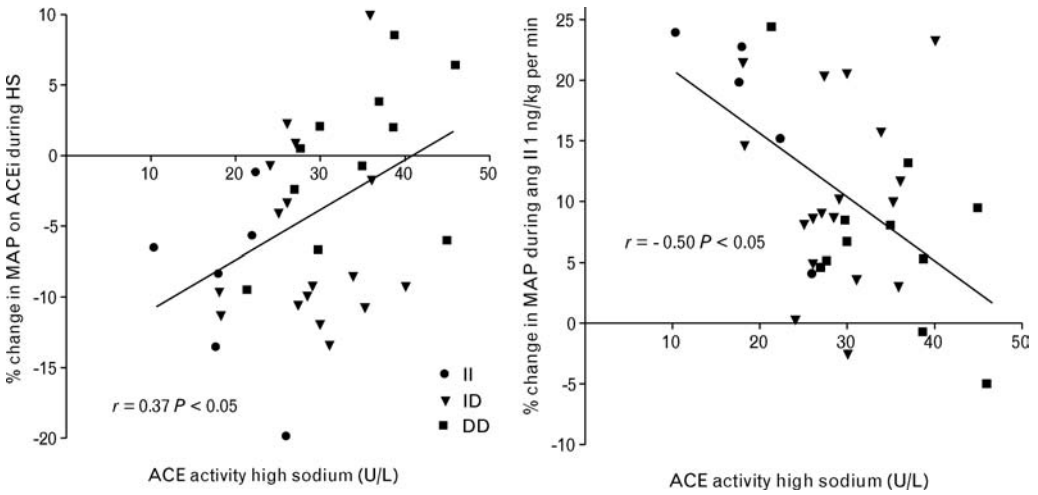
Serum ACE activity before treatment during high sodium correlated to the blood pressure response to ACEi during high sodium, with a better response in participants with lower ACE activities ($r = 0.37$, $P < 0.05$, Fig. 4, left

Table 3 Baseline plasma renin–angiotensin–aldosterone system parameters

	II	ID	DD
Low sodium diet			
ACE (U/l)	20.7 (16.9–26.0)	30.5 (26.3–37.3)††	36.2 (30.6–38.9)††
Angiotensin I (pmol/l)	35.2 (15.9–56.0)	17.7 (10.4–27.6)	35.2 (15.9–56.0)
Angiotensin II (pmol/l)	8.0 (3.4–13.3)	11.8 (9.2–16.6)	4.7 (1.1–10.5)
PRA (ng Ang I/ml per h)	6.8 (3.9–10.6)	5.8 (5.0–8.3)	5.8 (4.3–8.2)
Aldosterone (ng/l)	171 (77–260)	133 (94–167)	88 (68–174)
High sodium diet			
ACE (U/l)	20.0 (15.9–23.3)	28.7 (25.8–34.2)††	35.0 (27.7–38.8)††
Angiotensin I (pmol/l)	11.4 (7.5–50.8)	8.5 (4.7–14.4)$^{\#}$	10.0 (3.7–15.3)$^{\#}$
Angiotensin II (pmol/l)	2.0 (1.0–4.1)$^{\#}$	7.4 (1.7–12.0)$^{\#}$	3.3 (1.6–9.4)
PRA (ng Ang I/ml per h)	2.5 (1.7–3.1)$^{\#}$	2.2 (1.6–3.6)$^{\#}$	3.0 (1.2–4.0)$^{\#}$
Aldosterone (ng/l)	46 (34–67)$^{\#}$	45 (25–56)$^{\#}$	37 (22–78)$^{\#}$
Low sodium diet + ACE inhibition			
ACE (U/l)	2.1 (1.8–3.6)*	4.1 (2.8–5.2)*	4.0 (3.0–4.9)*††
Angiotensin I (pmol/l)	152.1 (70.5–234.5)*	106.3 (81.4–234.6)*	155.9 (72.4–211.0)*
Angiotensin II (pmol/l)	1.2 (0.9–5.7)*	5.9 (2.5–14.4)*	3.4 (1.7–6.6)
PRA (ng Ang I/ml per h)	32.2 (21.3–53.4)*	48.5 (35.5–61.6)*	48.3 (21.2–60.8)*
Aldosterone (ng/l)	124 (66–193)	96 (64–113)*	74 (55–129)
High sodium diet + ACE inhibition			
ACE (U/l)	2.0 (1.3–9.6)*	4.0 (2.8–7.4)*	4.0 (2.1–11.6)*
Angiotensin I (pmol/l)	27.5 (19.9–84.9)$^{*,\#}$	29.6 (9.2–59.7)$^{*,\#}$	33.5 (10.1–86.3)$^{*,\#}$
Angiotensin II (pmol/l)	2.5 (0.5–7.1)	8.1 (2.0–11.6)	0.5 (0.5–2.6)$^{\#}$
PRA (ng Ang I/ml per h)	10.0 (5.2–11.9)$^{*,\#}$	7.6 (4.8–14.0)$^{*,\#}$	7.5 (2.3–20.9)$^{*,\#}$
Aldosterone (ng/l)	43 (29–55)$^{\#}$	27 (20–39)$^{*,\#}$	30 (17–47)$^{\#}$

Medians and interquartile ranges are given. ACE, angiotensin-converting enzyme; PRA, plasma renin activity. $^{*}P < 0.05$, compared to placebo on same sodium diet. $^{\#}P < 0.05$, compared to high sodium. $^{\dagger}P < 0.05$, compared with II.

Fig. 4



Serum angiotensin-converting enzyme activity correlates with blood pressure response to ACEi and Ang II infusion. Correlation between serum activity and blood pressure response to ACEi during high sodium intake (left panel) and response to angiotensin II infusion during high salt + ACEi (right panel, 1 ng/kg per min). Spearman correlation coefficients are shown. ACEi, angiotensin-converting enzyme inhibition.

panel). This correlation was not present during low sodium. Serum ACE activity during high sodium before treatment also correlated to the pressor response to Ang II during treatment (Fig. 4, right panel, data for 1 ng/kg per min are presented). The response to Ang II was absent in participants with the highest ACE activity before treatment, but prominent in participants with lower ACE values before treatment ($r = -0.50$, $P < 0.05$). To account for possible confounding effects of differences in baseline blood pressure on the pressor response to Ang II multivariate analysis was performed. ACE genotype and ACE activity both predicted Ang II sensitivity to a similar extent. The relationship was not influenced by baseline blood pressure.

Discussion

This is the first study that prospectively demonstrates gene–environment interaction between the ACE I/D genotype and dietary sodium as regards therapy response to ACEi. High sodium intake blunts the blood pressure response to ACEi in DD homozygotes only; this resistance is ameliorated by low sodium intake. The resistance to ACEi is associated with a blunted pressor response to exogenous Ang II, consistent with higher activity of the endogenous tissue RAAS as a possible mechanism of therapy resistance; the latter is also corrected by low sodium intake.

Our current data, obtained by prospective modulation of sodium status, are in line with our prior retrospective, cross-sectional, findings in proteinuric patients [9], suggesting that the gene–environment interaction may be relevant in renal populations as well. Jacobsen *et al.* [22] discussed the importance of ACE I/D genotype in the renoprotective treatment of diabetic nephropathy and stressed the importance of individual nongenetic factors such as sodium intake. Unfortunately, many studies in renal patients do not consider sodium intake. It should be noted that the high sodium diet used here is not excessive, but moderately above average for the general population in the Netherlands. The finding that therapy responsiveness can be modified by dietary sodium is of potential clinical interest as it provides a strategy to circumvent genetically conferred therapy resistance.

As expected, during placebo serum ACE activity was highest in DD genotype, intermediate in ID and the lowest in II genotype, on either sodium intake. During treatment, serum ACE activity was adequately suppressed in all genotypes. In line with prior studies [23–25], other plasma RAAS parameters were not different either. However, circulating levels of RAAS components do not adequately reflect tissue RAAS activity, which is considered of more pathophysiological significance [26,27]. Unfortunately, tissue RAAS activity cannot be assessed directly in man. In rats, tissue ACE is induced by high sodium diet during ACEi, associated with persistence of vascular conversion of Ang I to Ang II despite ACEi and a blunted therapy response. Interestingly, low sodium reduced tissue ACE during ACEi and restored efficacy of inhibition of vascular ACE [28,29]. Boddi *et al.* [27] demonstrated that conversion of Ang I in the peripheral vascular bed was increased by high sodium diet in humans, but did not consider ACE genotype. Finally, high sodium increases the Ang I/Ang II ratio of responses

of blood pressure and aldosterone only in DD genotype [8]. Together with our current finding of a correlation between higher pretreatment ACE activity during high sodium with a worse response to ACEi and a blunted response to Ang II, these data raise the hypothesis that high sodium induces ACE activity at tissue level, in particular, in the DD genotype as a possible mechanism underlying a blunted response to ACEi during high sodium.

To our knowledge, this is the first in-vivo study showing an effect of ACE genotype on the pressor response to Ang II. The response was blunted in the DD genotype during ACEi combined with high sodium. This is consistent with in-vitro studies showing decreased vascular responsiveness to Ang II in human mammary arteries in DD genotype patients [30,31], without having documented sodium status. Low sodium, a state of RAAS activation, blunts the response to Ang II infusion [32,33], possibly due to increased local or circulating Ang II that downregulates AT-1 receptor expression [34,35], and/or receptor occupancy. The other way round, the Ang II response during sodium repletion is attributed to upregulation of AT1-receptors [36] reflecting suppression of RAAS activity and lower tissue Ang II. In our population as a whole, high sodium appropriately increased the Ang II pressor response. However, DD homozygotes did not have an appropriate Ang II response during ACEi on high sodium, suggesting persisting tissue RAAS activity in this condition, as a mechanism underlying their resistance to the effect of ACEi.

Prior studies, used infusion of Ang I as a tool to assess the functional effects of the ACE genotype-associated differences in ACE activity [8,24,37]. Ang I also leads to generation of Ang II by non-ACE pathways [38,39] that become relatively more important during ACEi, which makes the Ang I responses difficult to interpret during ACEi. As the vasoconstrictor response to Ang II can be used as an indirect marker for activity of the tissue RAAS [40], we used this tool to detect differences in endogenous RAAS activity between the genotypes during ACEi.

Our data suggest that consideration of sodium status as a relevant environmental factor may resolve several conflicting results on ACE genotype and the response to RAAS blockade in cardiovascular and renal disease. It has been pointed out that many association studies are flawed by methodological weaknesses, such as poor definition of the phenotype, inhomogeneous patient groups, selection bias by competing risks of the DD-genotype on cardiovascular mortality and lack of adjustment for cardiovascular and renal disease duration [11]. To provide proof of principle, we deliberately performed this study in healthy participants, and thus avoided disease heterogeneity and confounding by preexistent damage on therapy response [41,42]. Our study has some limitations. First, our study population was relatively small due to the very demanding study protocol. However, using each participant as his or her own control allowed to reliably assess the effect of change in sodium intake on the response parameters. Only six participants with the II genotype were included. This was anticipated from allele frequency and Hardy–Weinberg equilibrium. We refrained from enriching our population with II participants, as this might also lead to inadvertant selection for other unidentified factors. We studied only one dosage of ACEi, enalapril 20 mg once daily in the evening. When adequate suppression of serum ACE activity was obtained, it could have been of interest to study the effect of a supramaximal dosage of ACEi (or addition of other antihypertensives) as well. Finally, the fact that we studied healthy volunteers limits the generalizability to patient populations.

In conclusion, high sodium selectively blunts the blood pressure response to ACEi in DD genotype in healthy men, accompanied by a blunted pressor sensitivity to Ang II. Low sodium corrects both abnormalities. These data suggest that low sodium diet is important to obtain the therapeutic benefit of ACEi in DD homozygotes, but confirmation of our findings in patient populations is needed. If this gene–environment interaction would be present in patients, low sodium diet would be recommend not only to lower blood pressure as such, but also to overcome specific therapy resistance in ACE-DD participants.

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