The ACE (I/D) polymorphism and the RAAS in type 1 diabetes mellitus
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Chapter 5

Diabetes-associated increase in serum ACE level: functional consequences for pressor response to Angiotensin 1 infusion in relation to ACE (I/D) genotype.

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Submitted.
Abstract

**Aim/hypothesis.** Abnormalities in the renin angiotensin aldosterone system may be involved in the development of diabetic complications. Serum ACE level is increased in insulin-dependent diabetes mellitus but functional consequences are unknown.

**Methods.** To test possible functional consequences of the increase in serum ACE level, we compared the responses of blood pressure and renal hemodynamics to infusion of angiotensin (Ang) I in 22 uncomplicated type I diabetic patients with 22 healthy subjects, matched for the ACE (I/D) genotype. All subjects were studied twice, both after one week of low sodium intake (50 mmol/24h) and after one week of high sodium intake (200 mmol/24h), AngII responses were used as a reference.

**Results.** Diabetes, ACE-genotype and the interaction between these variables independently contributed to the serum ACE level, with the highest ACE level in diabetic DD-patients. During both sodium intakes, the blood pressure (MAP) response to infusion of AngI was more pronounced in diabetic patients ($P<0.05$). The decrease in ERPF was similar in both groups. The adaptation of angiotensin responsiveness to the altered sodium status was similar in diabetes and controls. On multivariate analysis, AngII responsiveness and both the presence of diabetes and ACE-genotype independently contributed to the MAP and ERPF responses to AngI. When serum ACE level was included in the model, diabetes and ACE-genotype no longer contributed to the AngI response of MAP, suggesting that the impact of both the diabetic state and the DD genotype on AngI responses is mediated by their effects on serum ACE level.

**Conclusions.** These data obtained in a pharmacological set-up suggest that the diabetes-associated increase in ACE level has functional consequences that are enhanced by the presence of the DD genotype. Further studies should explore possible pathophysiological consequences of this gene-environment interaction.

Introduction

The renin angiotensin aldosterone system (RAAS) is a main regulator of blood pressure and renal hemodynamics (1). Abnormalities in the RAAS have been implicated in the development of diabetic nephropathy, as suggested by the renoprotective effect of pharmacological blockade of the RAAS (2;3). Several abnormalities in the RAAS have been reported in insulin-dependent diabetes mellitus, such as altered plasma renin and/or
aldosterone levels (4;5) with a decreased aldosterone to renin-ratio (6). Furthermore, an altered responsiveness to angiotensin II (7;8) has been found, albeit not uniformly so (9-11). Finally, the serum level of angiotensin converting enzyme (ACE) is elevated in diabetes mellitus (12;13). However, the functional significance of these abnormalities, and their relative importance, is not well established.

Consequent to the elevated ACE level, one might expect enhanced conversion of angiotensin I in diabetic subjects. Whereas it is usually assumed that ACE is not rate-limiting for the generation of AngII (14), this notion is challenged by the increased response to AngI in subjects with genetically increased ACE levels, associated with the ACE insertion/deletion (I/D) genotype (15;16). The mechanisms and functional consequences of diabetes-associated increases in ACE levels, however, may not be similar to those of genetically determined elevations in plasma ACE levels. It has been suggested that high ACE levels in diabetes reflect mere shedding of ACE from cell membranes, due to impaired endothelial anchoring of this ecto-enzyme, as a feature of endothelial dysfunction (13), whereas the genetically elevated ACE level in DD genotype reflect elevated tissue ACE as well (17).

We recently reported the effect of ACE-genotype on responses to AngI in normal subjects (16) and uncomplicated type I diabetic subjects (6), studied according to parallel designed experimental protocols. Consequently, these data allow comparison of AngI responses in healthy controls and diabetic subjects, enabling us to test the hypothesis that the elevated ACE level in DM is associated with an increased AngI response. The purpose of the present study, therefore, is to analyse the functional impact of the diabetes-associated elevation in serum ACE level, by comparing AngI responses of blood pressure and renal hemodynamics between uncomplicated type I diabetic patients and healthy subjects. Responses to AngII were used as a reference. As sodium status can affect AngI conversion (18) as well as AngII sensitivity (19;20), the experiments were carried out during low as well as liberal sodium diet. As ACE-genotype affects AngI responses, this requires matching the diabetic and healthy subjects for ACE-genotype. From the original populations, therefore, matching subjects were selected and additional subjects, prospectively genotyped, were included to allow a valid comparison between the groups.

Materials and Methods

The original study populations from the two previous studies have been described elsewhere.
To obtain a proper match for ACE-genotype, we included 6 additional healthy subjects, 5 II-homozygotes and 1 DD-homozygote. Thus, we included 22 diabetic and 22 healthy subjects, with an individual match for age (within 5 years), gender, body mass index (BMI, within 3 kg/m²) and ACE-genotype (II DD and II II in both groups). Eligible subjects had normal findings on physical examination and no previous medical history of cardiovascular disease. Exclusion criteria were an increased blood pressure (BP >140/90 mmHg), renal function impairment (serum creatinine >106 μmol/l) and obesity (BMI > 30kg/m²). Diabetic patients were considered insulin-dependent because of ketosis-prone diabetes and an onset of disease before the age of 35 yr. All patients had a diabetes duration of at least 2 yr and were normoalbuminuric (UalbV < 30mg/24h). They did not use other medication than insulin. Poor metabolic control (HbA1c level > 8.5%), clinically manifest neuropathy (defined by symptoms and/or abnormal tests of sensation) and proliferative diabetic retinopathy were additional exclusion criteria for the diabetic patients. Background retinopathy was present in 3/22 diabetic patients.

**Study design**

All participants were studied on two occasions, after they had adhered to a low (50 mmol sodium/day) and a liberal (200 mmol/day) sodium diet. The diets were applied in randomized order, after instruction by an experienced dietician. For each study day, subjects were instructed to maintain the prescribed sodium diet during the 7 days preceding the study day. Potassium intake was standardized at 100 mmol/day. During the diet patients were ambulant, continued their daily activities and refrained from heavy exercise. Compliance to the prescribed sodium intake was checked 3 days before each study day by 24h urine collections, in order to ensure inclusion only when dietary compliance was adequate. In addition, 24h urine was collected the last day before each study day: these values are given in Table 1.

The studies were started at 8.00 am, after an overnight fast, having refrained from alcohol and caffeine containing drinks for 12h. Intravenous catheters were inserted into each forearm for infusion and drawing of blood samples. During the whole study, the participants remained in semirecumbent position in a quiet room. All subjects ingested 250 ml of oral fluids each hour and a small meal of similar caloric content each two hours. Sodium intake during the study day was adjusted according to the prescribed diet. To ensure sufficient urine output, glucose 5% (250 ml/hr) was administered in the right antecubital vein. The ensuing waterloading is not expected to suppress RAAS parameters to a relevant extent, in contrast to water and saline loading (21;22).
The diabetic patients received a low dose insulin infusion (30 mU/kg/h) and blood glucose was kept at 5 mmol/l by varying the infusion rate of a glucose infusion (dextrose 20% to which 20ml/l KCl was added to prevent hypokalemia), in order to minimize the effects of variations in glycemia on systemic and renal haemodynamics (23;24). To this purpose blood glucose was measured every 10 min using an APEC glucose analyser (APEC Inc, Danvers, MA, USA). With this regimen, peripheral insulin levels are stable at approximately 30 mU/l (25). Each study day, sodium intake was adjusted according to the prescribed sodium diet. The first two hours (from 8 to 10 am) served for equilibration of the renal function tracer, and for stabilization of blood glucose levels.

**Blood pressure.**

Blood pressure (BP) was measured with a semi-automated device (Dinamap® 1846, Critikon, Tampa, FL, USA) at 15 minute intervals, except during the angiotensin infusions when blood pressure was recorded every 5 minutes. Mean arterial pressure (MAP) was calculated as diastolic blood pressure plus one-third of the pulse pressure (mmHg).

**Renal function measurements.**

Effective renal plasma flow (ERPF) was measured as the clearance of a constantly infused tracer dose of $^{131}$I-hippuran, as previously described (26). ERPF is calculated as the plasma clearance of $^{131}$I-hippuran from the formula $(I\times V)/P$ after steady state plasma levels of the tracer have been obtained. In this formula, I stands for the concentration of tracer in the infusion fluid, V for the infusion rate (volume) and P for the obtained plasma tracer level. This precludes bias due to urine collection errors, as it has been shown that with perfect urine collection, during steady state plasma tracer levels, the urinary clearance of $^{131}$I-hippuran equals its plasma clearance (26;27). Blood samples for determination of tracer plasma levels were drawn hourly, from 10am to 6pm. ERPF was corrected for 1.73m$^2$ of body surface area. This method has a day-to-day variation coefficient of 5% for ERPF (26).

**Angiotensin infusions**

Baseline values for blood pressure and renal hemodynamics were obtained from 10 to 12 am. From 12.00 am to 02.00 pm, AngI (Clinalfa AG, Switzerland) was administered in the left antecubital vein in a dose of 4 ng/kg/h in the first hour and 8 ng/kg/h in the
second hour. After a washout period from 02.00 pm to 04.00 pm, AngII (Clinalfa AG, Switzerland) was administered from 04.00 pm to 06.00 pm in a dose of 4 ng/kg/h in the first hour and 8 ng/kg/h in the second hour.

**Laboratory measurements.**

Venous blood samples were taken at the end of each clearance period. Blood was drawn from an intravenous catheter inserted into the right antecubital vein. Blood samples were immediately centrifuged at 4°C and the samples were stored at -20°C before assay. Serum ACE activity was determined with an HPLC-assisted assay, as the generation of Hip from the substrate Hip-His-Leu. Normal values in our laboratory range from 10-40 U/l (28). Plasma renin activity was determined with a commercially available double-antibody RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Glycated hemoglobin was measured by HPLC (Bio-Rad, Veenendaal, The Netherlands; normal range 4.6-6.1%).

**Statistical analysis and calculations.**

Data are given as mean ± SD. The average of the BP values measured from 10-12 am (at 15-min intervals) were used as baseline BP values. The percent change in MAP during a given infusion step of AngI was analysed as the average of all MAP values measured during the 1-h infusion period (at 5-min intervals). The average of the BP values measured from 3-4 pm (at 15-min intervals) were used as recovery values, to which the BP responses to AngII infusion (at 5-min intervals during the both infusion steps) were related for calculation of percent changes. The variation in the sequential blood pressures was below 5% for each different time interval during the study day. For renal hemodynamics, the average values of the 2-h baseline clearance period (10-12 am) were used as baseline values ANOVA with post-hoc Bonferroni correction was used to analyse the MAP and ERPF values obtained during both study days within and between both groups. Student t-tests were used for other between groups comparisons. To test whether the diabetic state and the ACE-genotype were independent determinants of serum ACE level, and to determine possible interactions between these variables, we performed multiple regression analysis, with serum ACE as the dependent variable and diabetes, ACE-genotype and the interaction between these two variables as independent variables. Multiple regression analysis was also conducted using linear regression models to analyze for the determinants of the systemic and renal responses to infusion of AngI. Independent variables tested were the presence of diabetes,
Table 1. Clinical and laboratory characteristics during high (HS, 200 mmol sodium per day) and low (LS, 50 mmol sodium per day) sodium intake in type I diabetic patients and healthy control subjects.

<table>
<thead>
<tr>
<th></th>
<th>Type I DM</th>
<th>Healthy subjects</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (M:F)</td>
<td>22 (13:9)</td>
<td>22 (13:9)</td>
<td>NS</td>
</tr>
<tr>
<td>Age (yr.)</td>
<td>28.2 ± 6</td>
<td>25.5 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes duration (yr.)</td>
<td>12.3 ± 5</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.8 ± 2.6</td>
<td>21.3 ± 3.1</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Body weight (kg) HS</td>
<td>77.7 ± 12.3</td>
<td>73.0 ± 13.3</td>
<td>NS</td>
</tr>
<tr>
<td>Body weight (kg) LS</td>
<td>77.1 ± 12.3</td>
<td>71.7 ± 13.5</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.3 ± 0.5</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>UNaV (mmol/24hr) HS</td>
<td>246 ± 71</td>
<td>246 ± 52</td>
<td>NS</td>
</tr>
<tr>
<td>UNaV (mmol/24hr) LS</td>
<td>40 ± 16</td>
<td>50 ± 29</td>
<td>NS</td>
</tr>
<tr>
<td>ACE (U/l) HS II / DD</td>
<td>25 ± 4 / 46 ± 14</td>
<td>20 ± 5 / 27 ± 5</td>
<td>P&lt;0.05 (II and DD)</td>
</tr>
<tr>
<td>ACE (U/l) LS II / DD</td>
<td>26 ± 4 / 45 ± 14</td>
<td>19 ± 5 / 28 ± 6</td>
<td>P&lt;0.001 (II and DD)</td>
</tr>
</tbody>
</table>

* The mean ± SD is given. + P<0.001 vs. HS. † P<0.05 vs. HS. UNaV: urinary sodium excretion.

ACE-genotype, sodium status (categorical variables) and AngII sensitivity (as the percentage increase to infusion of AngII in the same person). Two-sided P-values less than 0.05 were considered to be significant.

Results

Patient characteristics are given in table 1. ACE levels were significantly higher in the diabetic patients (P<0.001) and were not affected by sodium intake. A division according to ACE-genotype showed that the difference in ACE level between diabetic (DM) and healthy subjects (C) was more pronounced in the DD-genotype (fig.1). On multiple regression analysis (r² 0.63, P<0.001), both the presence of DM (P<0.001) and ACE-genotype (P<0.001) were independent determinants of circulating ACE levels. Moreover, a significant interaction between the diabetic state and the ACE-genotype was observed (p<0.01), indicating that the combination of DM and DD genotype was associated with a significantly more elevated serum ACE level.

No further genotype associated differences in patient characteristics were found,
Table 2. Multiple regression analysis of AngI sensitivity.

**Models without serum ACE level**

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Determinant</th>
<th>Model r²/ P</th>
<th>Part.corr.coeff.</th>
<th>P-value</th>
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<tbody>
<tr>
<td>ΔMAP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8ng/kg/min</td>
<td>Diabetes</td>
<td>0.282/0.001</td>
<td>0.312</td>
<td>0.004</td>
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<td>Sodium intake</td>
<td>0.366</td>
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<td>0.001</td>
</tr>
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<td>ACE-genotype</td>
<td>0.264</td>
<td></td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>AngII-sensitivity</td>
<td>0.263</td>
<td></td>
<td>0.015</td>
</tr>
<tr>
<td>ΔERPF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8ng/kg/min</td>
<td>Diabetes</td>
<td>0.367/0.001</td>
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<td>0.736</td>
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<td></td>
<td>Sodium intake</td>
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<td>0.001</td>
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<tr>
<td></td>
<td>ACE-genotype</td>
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<td></td>
<td>0.023</td>
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<td></td>
<td>AngII-sensitivity</td>
<td>0.453</td>
<td></td>
<td>0.001</td>
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**Models with serum ACE level**

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Determinant</th>
<th>Model r²/ P</th>
<th>Part.corr.coeff.</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>ΔMAP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8ng/kg/min</td>
<td>Diabetes</td>
<td>0.307/0.001</td>
<td>0.134</td>
<td>0.224</td>
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<tr>
<td></td>
<td>Sodium intake</td>
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<td>0.001</td>
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<tr>
<td></td>
<td>ACE-genotype</td>
<td>0.077</td>
<td></td>
<td>0.489</td>
</tr>
<tr>
<td></td>
<td>AngII-sensitivity</td>
<td>0.279</td>
<td></td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Serum ACE level</td>
<td>0.216</td>
<td></td>
<td>0.049</td>
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<tr>
<td>ΔERPF</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8ng/kg/min</td>
<td>Diabetes</td>
<td>0.361/0.001</td>
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<td>0.604</td>
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<tr>
<td></td>
<td>Sodium intake</td>
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<td>ACE-genotype</td>
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<td>0.045</td>
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<td></td>
<td>AngII-sensitivity</td>
<td>0.455</td>
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<td>0.001</td>
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<tr>
<td></td>
<td>Serum ACE level</td>
<td>0.046</td>
<td></td>
<td>0.678</td>
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</table>

Part.corr.coeff. = Partial correlation coefficient.
therefore, the other characteristics are presented for both genotypes together. Dietary compliance was adequate in both groups, as shown by 24h sodium excretions. Body weight was significantly higher during high as compared to low sodium intake in both groups. Baseline MAP (low sodium DM 88 ± 8, C 88 ± 7 mmHg, NS; high sodium DM 90 ± 8, C 88 ± 6 mmHg, NS, fig.2) and ERPF (low sodium DM 493 ± 58, C 510 ± 53 ml/min/1.73m², NS; high sodium DM 537 ± 71, C 539 ± 64 ml/min/1.73m², NS, fig.2) were similar in diabetic patients and controls during both sodium intakes. The high sodium sodium intake increased baseline blood pressure in diabetic patients only (p<0.05, fig.2). ERPF was higher during high sodium intake in both groups (P<0.001, fig.2).

Mean values for MAP and ERPF before and during infusion of AngI and AngII during both study days are given in figure 2. During both ang infusions a significant, dose-dependent rise in MAP and a fall in ERPF occured in diabetic and healthy subjects during low and liberal sodium intake. For blood pressure, the 2h recovery period resulted in a complete recovery to baseline values in both groups. ERPF values were slightly lower after recovery compared to baseline in both groups during both sodium intakes, but the difference did not reach statistical significance. During high sodium the blood pressure levels achieved during the infusion of AngI and II were higher in the diabetic patients compared to the healthy subjects (fig.2B). During low sodium, MAP was similar in both groups during both infusion steps (fig.2A). ERPF was similar in both groups at baseline and during infusion of AngI and II, during both study days (fig.2C/D).

The changes in MAP and ERPF during infusion of AngI and AngII in both groups are given in figure 3. First, it shows that all responses were more pronounced during HS in healthy as well as diabetic subjects. During both sodium intakes, the increase in MAP in
Figure 2. Blood pressure (MAP) and renal hemodynamic (ERPF) values obtained during baseline, Ang I infusion, recovery and Ang II infusion in diabetic patients (DM) and healthy subjects during low sodium and high sodium intake. Data are mean ± SEM, all values obtained during infusion of Ang I and Ang II were significantly different from baseline (P < 0.01).

response to Ang I was significantly more pronounced in the diabetic patients compared to healthy subjects. The Ang II responses were similar in both groups during low sodium, whereas during high sodium the Ang II response was more pronounced in DM (fig.3). The decrease in ERPF by Ang I and Ang II was similar in both groups, during both sodium intakes (fig.3).

To analyze the relative impact of the diabetic state, sodium status, ACE-genotype and Ang II sensitivity on the responsiveness of blood pressure and ERPF to Ang I, we conducted a multiple linear regression analysis. With respect to the blood pressure response, it was found that diabetes, high sodium intake, the DD-genotype and Ang II-sensitivity were independently, and positively associated with Ang I response (table 2). For the ERPF response, diabetes was not associated with an increased response to infusion of Ang I, but sodium intake, DD-genotype and Ang II sensitivity all contributed to an increased response
to AngI (table 2). When serum ACE-level was included in the blood pressure model, both diabetes and ACE-genotype lost their significance, whereas serum ACE level (model $r^2 = 0.31$, partial correlation 0.22, $P<0.05$) independently contributed to the AngI-induced blood pressure response, together with sodium intake and AngII sensitivity. This suggests that the independent contributions of diabetes and ACE-genotype on the AngI response can be explained by their elevating effects on serum ACE level. No effects of serum ACE level were found for the response of ERPF (table 2).

**Discussion**

In this study serum ACE level was elevated in normotensive normoalbuminuric diabetic patients. Interestingly, the diabetes-associated increase in serum ACE level was more pronounced in patients carrying the DD-genotype, suggesting a gene-environment interaction between diabetes and ACE-genotype on circulating ACE. The blood pressure response to AngI was higher in diabetic patients than in healthy subjects during both sodium intakes, whereas no differences in the ERPF response were observed between diabetic patients and healthy subjects. On multivariate analysis both the presence of diabetes and the ACE-genotype independently contributed to the pressor response to AngI.
When ACE level was included in the model, the diabetic state and the ACE-genotype did no longer significantly influence the AngI response of blood pressure, strongly suggesting that the impact of these two factors on AngI response is mediated by their effects on ACE level. Finally, the sodium-induced shift of the responsiveness of blood pressure and ERPF to angiotensins was similar in diabetic patients and healthy subjects.

Diabetes mellitus has been reported to be associated with increased serum ACE levels (12;13), but its functional consequences are largely unknown. Some authors found ACE levels to be elevated mainly in association with signs of target organ damage or in relation to poor metabolic control (13). From the association with micro-angiopathy (29) it was suggested that the elevated ACE levels could be a marker of endothelial damage. In the diabetic patients studied here, target organ damage was virtually absent, as apparent from the normo-albuminuric state, whereas background retinopathy was present in only 3/22 patients. Others found that serum ACE levels were neither related to diabetes duration, nor to diabetic retinopathy severity, findings that do not fit with the concept of microangiopathy as the cause of elevated ACE levels (30). An increased shedding of ACE from the endothelium has been shown in families carrying a mutation of the ACE gene that results in remarkably increased (5-fold) plasma ACE-levels (31). Cell-bound ACE is unaltered in these subjects and they do not have clinical abnormalities (32). To our knowledge, it is unknown whether diabetes is associated with increased cell-bound ACE. Furthermore, no information is available on the relation between the ACE genoetype and the increased serum ACE levels in diabetic patients. It has been suggested that the increase in ACE level in a sample of diabetic patients with nephropathy was due to an overrepresentation of the D-allele (33). In the current paper, we found that an increased ACE level in diabetic patients is present in the absence of diabetes related complications. This indicates that the diabetic state as such is responsible for the increased serum ACE levels instead of the diabetes-associated microvascular damage.

Interestingly, the diabetes related increase in serum ACE level was more pronounced in DD-patients, suggesting that the ACE-genotype seems to be an important determinant of the serum ACE level that ultimately results when the diabetic state induces a rise in ACE level. In line with our data, others reported that the CABG induced increase in serum ACE level (probably due to acute endothelial damage) was also more pronounced in DD-homozygotes (34). This suggests that the genetic background, i.e. the ACE-genotype, determines the magnitude of the increase in ACE in response to an environmental trigger, such as diabetes. It would be of great interest to know whether this also applies to the regulation of tissue-ACE, but so far no data are available on this issue.
In line with the ACE levels, we also found that AngI pressor responses were enhanced by both the presence of diabetes and the DD genotype. In our analysis of the AngI response, AngII response was used as a reference, as AngII responsiveness is an important determinant of AngI responses, as confirmed by our present data. A word of caution is needed here, as in our study the order of AngI and AngII infusions was not randomized. Therefore, we cannot exclude the possibility that the AngII responses were affected by the preceding AngI infusions, which warrants for caution as to a strict quantitative comparison of AngI and AngII responses. This restriction taken in mind, however, the data on AngI responses support the assumption that the elevated ACE level has functional consequences, at least in the present experimental set-up using pharmacological doses of angiotensin. Whether the functional consequences extend to clinical (patho-)physiological conditions cannot be derived from our data.

Our data confirm the importance of AngII responsiveness as a main determinant of the responses of blood pressure and renal function to AngI, and allow several additional conclusions on the function of the RAAS in diabetes. First, in line with other reports (7;9), we found an enhanced pressor responsiveness to both AngI and AngII in diabetes. Our data are the first to show that this occurs irrespective of sodium intake and that, accordingly, the sodium-induced shift in angiotensin-sensitivity is normal in diabetes, albeit at a higher level.

At variance with the increased pressor responses, the responses of ERPF to angiotensins were normal in the diabetic patients, again irrespective of sodium intake, with a normal shift in sensitivity from low to high sodium intake. Several studies investigated blood pressure responses to AngII in type I diabetes, reporting either an enhanced pressor response (7) or an enhanced response in subjects with target organ damage only (9). Studies on renal hemodynamics reported normal responses (10;11) or a tendency towards blunted responses (8). These studies, however, were not standardized for sodium intake and the studied patients were not comparable with respect to microvascular complications. Our study is the first to document a dissociation between an enhanced blood pressure response and a normal response of ERPF, irrespective sodium intake, in well controlled type I diabetic patients without signs of target organ damage. Our present findings display a striking similarity with our previous report on the responses to norepinephrine, i.e an enhanced systemic but normal renal vasoconstrictor sensitivity in diabetic subjects (25). The enhanced systemic pressor responsiveness in diabetes might be due to an impaired capacity to counteract the pressor effect of exogenous agents by, for instance, impaired baroreceptor sensitivity (35), diminished parasympathetic function (36) and impairment of endothelial function (37).
The diabetic subjects were tested using euglycemic clamp, with low dose insulin infusion. Insulin is known to stimulate sodium retention, vasodilation and sympathetic activity (38-42), which could have affected our results. However, refraining from the euglycemic clamp condition would have most likely exerted more bias in renal function. It is known that hyperglycemia affects renal hemodynamics, most likely resulting from RAAS activation (23;24;43;44). The low dose of insulin that we used provides stable peripheral insulin concentrations that amount to approximately 30 mU/l (25), i.e. slightly below the average insulin concentrations during daily life in diabetic patients. Therefore, our experimental conditions during the renal function studies more or less mimic those in fairly well-regulated diabetic patients during daily life. The healthy control subjects did not receive insulin infusion, as insulin may exert effects on renal hemodynamics (45). Thus, as inevitable in studies comparing diabetic patients and healthy subjects, study conditions did not completely match for diabetic patients and healthy subjects. This limitation has to be taken in mind in the interpretation of the data.

In conclusion, the diabetes-associated increase in serum ACE level is more pronounced in the DD-genotype. The blood pressure responses, but not the responses of ERPF to AngI were enhanced in normotensive, normoalbuminuric diabetic patients as compared to healthy subjects, irrespective sodium intake. The increased AngI responses in DM could not be accounted for by the increased responsiveness to AngII, as for a given AngII response both DM and ACE-genotype were independent determinants for the AngI response. Their effects are likely to be mediated by serum ACE level, as supported by the multivariate model. Thus, in diabetic patients ACE level as well as pressor responses to pharmacological doses of AngI are determined by both the environmental factor diabetes, and the genetic factor ACE (I/D) polymorphism. Further studies should explore the (patho-) physiological consequences of this gene-environment interaction.
References


(16) van der Kleij FG, de Jong PE, Henning RH, de Zeeuw D, Navis G. Enhanced Responses of Blood Pressure, Renal Function, and Aldosterone to Angiotensin I in the DD Genotype Are Blunted by Low


