The ACE (I/D) polymorphism and the RAAS in type 1 diabetes mellitus
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Chapter 3

Short-term moderate sodium restriction induces relative hyperfiltration in normotensive normoalbuminuric type 1 diabetes mellitus.

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Abstract

Aim/hypothesis. Type 1 diabetes mellitus is associated with an increased extracellular volume. Sodium restriction would seem a logical measure, but literature on its renal effects is conflicting. We therefore studied the effects of sodium restriction on renal hemodynamics in uncomplicated type 1 diabetes mellitus and controls.

Methods. 24 uncomplicated type 1 diabetic patients and 24 matched controls were studied twice in random order: after a week of 50 mmol or after 200 mmol sodium intake, respectively. The diabetic patients were studied under normoglycemic clamp conditions. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured as the clearances of iothalamate and hippuran, respectively.

Results. During liberal sodium intake, GFR, ERPF and filtration fraction (FF) were similar in the diabetic patients and the controls. Sodium restriction decreased the effective renal plasma flow in both groups, whereas glomerular filtration rate only decreased in the control subjects. Consequently, in the diabetic patients, FF was significantly increased on low sodium (4.1 ± 8.4%, p<0.05 vs. liberal sodium). As a consequence, FF (24.0 ± 2.6 vs 22.1 ± 2.0%, p<0.05) and GFR (119 ± 14 vs 110 ± 13 ml/min, p<0.05) were significantly higher in diabetic subjects than in controls during sodium restriction.

Conclusions. Short-term moderate sodium restriction induces relative hyperfiltration in uncomplicated type 1 diabetes mellitus. This may indicate an increased intraglomerular pressure. Sodium restriction may be an unfavorable preventive approach in diabetes mellitus, but its long-term effects remain to be studied.

Introduction

Diabetic nephropathy is one of the most serious complications of diabetes mellitus. Nephropathy develops in approximately 35% of diabetic patients (1). Preventive measures include good metabolic control and rigorous antihypertensive treatment, preferably by renin-angiotensin system (RAS) blocking agents (2). Early abnormalities preceding overt nephropathy include microalbuminuria, a rise in blood pressure and an increase in intraglomerular pressure (3;4). Volume expansion is likely to be relevant in these processes, as renal sodium excretion is known to be blunted in diabetic patients (5-9), an effect that might be mediated by the sodium retaining effects of insulin (10;11).
Considering the abnormalities in extracellular volume, dietary sodium restriction would seem a logical measure. However, low sodium intake activates the RAS (12;13) and an increase in RAS-activity has been proposed to play a role in the development of diabetic nephropathy, as suggested by the preventive effect of ACE-inhibitors on the development of diabetic nephropathy (2;14). Thus, sodium restriction could theoretically exert unfavorable effects in the diabetic kidney.

Reports about the renal effects of sodium restriction in diabetes mellitus (DM) are surprisingly scarce, and data are conflicting. In experimental diabetes in the streptozotocin/rat model, both the augmented renal plasma flow (RPF) and glomerular hyperfiltration were improved by sodium restriction (15-17). On the other hand, an increase in RPF and glomerular filtration rate (GFR) with sodium restriction has been reported (18). In man, two small studies are available so far, using a rigorous restriction in dietary sodium (i.e. to 20 mmol/day), yielding conflicting results (19;20). Thus, so far data are hard to interpret.

It would therefore be relevant to investigate the renal effects of a dietary sodium restriction that is feasible in clinical practice. In the present study, therefore, we evaluated the renal hemodynamic effects of 50 mmol versus a 200 mmol sodium intake in uncomplicated type 1 diabetic patients and healthy control subjects.

Materials and Methods

Subjects and study design

Twenty-four normotensive (systolic pressure < 140 mmHg, diastolic pressure < 85 mmHg), normo-albuminuric (<30 mg/24hr) type 1 diabetic patients were compared with 24 healthy control subjects, according to a parallel open-label randomized cross-over design. Participants were matched for age (within 3 years), sex (M/F), and body mass index (<3 kg/m², BMI was calculated as weight (kg) divided by height (m) squared and expressed in kg/m²). All diabetic patients suffered from ketosis prone diabetes mellitus and their age of onset was < 35 years of age. Metabolic control was adequate in diabetic patients, as indicated by an HbA1c concentration < 8.0% in all. The diabetic patients received an average of 61 ± 18 units of insulin during the days before the study was performed, using modern insulin schemes consisting of long-acting insulin before the night and 3 injections of short-acting insulin before the meals. The study was approved by the local Medical Ethical Committee and all participants gave written informed consent.
The participants were studied twice, and counseled by a research dietician who advised a low sodium diet (50 mmol sodium per day) and a liberal sodium diet (200 mmol sodium per day). The sequence of the diets randomized by drawing an allocation number from closed envelopes. The diet periods were separated by at least one week (range 10-17 days) to rule out carry-over effects. Both diets were normocaloric. Diets were started seven days prior to each day of investigation. During the diet periods the subjects were ambulant and continued their normal daily activities. The low and liberal sodium diets were randomized and adherence was checked by measuring sodium excretion in 24h urine collections on the third or fourth day of the diet, as well as on the day prior to the study day.

**Experiments**

On each study day the subjects reported at the hospital research unit at 7.30 after a fast, having refrained from food, alcohol, drinking and strenuous exercise for 12 hours. The experiments started at 8.00 AM. Two 18-gauge peripheral venous cannulas were inserted into an antecubital vein of the left and right arm for infusion of isotopes, glucose and insulin and for drawing of blood samples. During the experiment subjects remained in the semisupine position in a quiet room. They drank 250 ml of drinks without caffeine each hour. Smoking was not allowed during the study day.

The diabetic patients were studied using the euglycemic clamp technique, whereby normoglycemic conditions (blood glucose 5.0 mmol/l) were attained using a low insulin infusion (30 mU/kg/hr) with a variable glucose infusion (dextrose 20% to which 20ml/l KCl was added to prevent hypokalemia). There were no differences in the amount of glucose required to maintain euglycemia during low and liberal sodium intake. The healthy subjects were not studied using this technique, assuming a normal blood glucose regulation.

After two hours of equilibration, blood was sampled in prechilled tubes hourly, during two consecutive hours. Blood samples were centrifuged at 3000 rpm. at 4 °C. Urine was collected after blood samples were taken. Blood pressure was measured using an automated device (Dinamap®, Criticon, Tampa, Florida, USA) at 15 min. intervals. Hormonal parameters were measured at 10.00 AM and 12.00 AM and the average values of both measurements were used for analysis.

**Renal function measurements**

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured by
constant infusion of radiolabelled tracers, $^{125}$I-iothalamate and $^{131}$I-hippurate, respectively, as previously described (21). After drawing a blank blood sample, a priming solution containing 0.4 ml/kg body weight of the infusion solution (0.04 MBq of $^{125}$I-iothalamate and 0.03 MBq of $^{131}$I-hippurate) plus an extra of 0.6 MBq of $^{125}$I-iothalamate was given at 8 am, followed by infusion at 12 ml/h. In order to attain stable plasma levels of both tracers, a two hour stabilization period followed, after which baseline measurements started at 10 am. The clearances were calculated as $(U^*V)/P$ and $(I^*V)/P$, respectively. $U^*V$ represents the urinary excretion of the tracer, $I^*V$ represents the infusion rate of the tracer; $P$ represents the tracer value in plasma at the end of each clearance period. This method corrects for incomplete bladder emptying and dead space, by multiplying the urinary clearance of $^{125}$I-iothalamate with the ratio of the plasma and urinary clearance of $^{131}$I-hippuran (21). The filtration fraction (FF) was calculated as the ratio of GFR and ERPF and expressed as percentage. Renal vascular resistance (RVR) was calculated as MAP divided by ERPF. GFR and ERPF were corrected for $1.73m^2$ of body surface area. This method has a day-to-day variation coefficient of 2.5% for GFR and 5% for ERPF (21).

Laboratory methods

Serum electrolytes, creatinine, liver enzymes and blood count were determined by an automated multi-analyzer (MEGA®, Merck, Darmstadt, Germany). Plasma glucose concentrations were measured using the APEC glucose analyzer (APEC®, Danvers, Massachusetts, USA). Plasma renin activity (PRA) was measured using an in-home radioimmunoassay. HbA1c was measured by high-performance liquid chromatography (Bio-Rad, Veenendaal, The Netherlands; normal range 4.6-6.1%).

Statistical analysis

A power-analysis was conducted, based on glomerular filtration rate (GFR) as well as filtration fraction (FF) (i.e: both parameters for hyperfiltration) as primary end-points. For both GFR and FF, in our hands, the SD of the population is approximately 10%. Thus, our study was powered to detect a 10% difference in GFR and FF between the groups, with an $\alpha$ of 0.05 and a $\beta$ of 0.90 with 22 subjects per groups. Twenty-four subjects were included in both groups to allow for possible drop-outs. Secondary end-points were blood pressure and renal plasma flow (ERPF).

Data are expressed as mean ± SD and 95% confidence intervals. Unpaired student
Table 1. Clinical characteristics, urinary sodium excretion, blood glucose during liberal and low sodium intake in Type I diabetic patients and healthy control subjects

<table>
<thead>
<tr>
<th></th>
<th>Type I DM patients</th>
<th>Control subjects</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>24</td>
<td>24</td>
<td>NS</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>28.2 ± 6</td>
<td>25.1 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of DM (yr)</td>
<td>12.3 ± 6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age of onset (yr)</td>
<td>15.8 ± 7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Body weight LS (kg)</td>
<td>75.3 ± 9.7</td>
<td>72.1 ± 13.1</td>
<td>NS</td>
</tr>
<tr>
<td>Body weight HS (kg)</td>
<td>76.1 ± 9.9</td>
<td>73.3 ± 12.9</td>
<td>NS</td>
</tr>
<tr>
<td>BMI LS (kg/m²)</td>
<td>23.5 ± 2.2</td>
<td>21.8 ± 3.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BMI HS (kg/m²)</td>
<td>23.7 ± 2.2</td>
<td>22.2 ± 3.3</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%) during LS</td>
<td>7.4 ± 0.5</td>
<td>5.2 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%) during HS</td>
<td>7.4 ± 0.6</td>
<td>5.2 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urinary sodium excretion during LS (mmol/24hr)</td>
<td>37.6 ± 13.1</td>
<td>45.4 ± 28.2</td>
<td>NS</td>
</tr>
<tr>
<td>Urinary sodium excretion during HS (mmol/24hr)</td>
<td>249.4 ± 70.7</td>
<td>253.5 ± 58.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

*The mean ± standard deviation is given. b Non-significant vs LS. c P<0.001 vs LS.

Table 2. Blood pressure and renal hemodynamics during low (LS) and liberal (HS) sodium intake in Type I diabetic patients (DM) and healthy control subjects (C)

<table>
<thead>
<tr>
<th></th>
<th>DM HS</th>
<th>DM LS</th>
<th>C HS</th>
<th>C LS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>90 ± 8</td>
<td>87 ± 7</td>
<td>89 ± 7</td>
<td>88 ± 7</td>
</tr>
<tr>
<td>GFR (ml/min/1.73m²)</td>
<td>123 ± 10</td>
<td>119 ± 14</td>
<td>120 ± 18</td>
<td>110 ± 13 b</td>
</tr>
<tr>
<td>ERPF (ml/min/1.73m²)</td>
<td>538 ± 77</td>
<td>498 ± 59 b</td>
<td>539 ± 68</td>
<td>502 ± 58 b</td>
</tr>
<tr>
<td>FF (%)</td>
<td>23.1 ± 2.6</td>
<td>24.0 ± 2.6 cd</td>
<td>22.5 ± 2.9</td>
<td>22.1 ± 2.0</td>
</tr>
<tr>
<td>RVR (mmHg/L’min)</td>
<td>154 ± 3</td>
<td>178 ± 3 b</td>
<td>151 ± 2</td>
<td>177 ± 3 b</td>
</tr>
</tbody>
</table>

*The mean ± standard deviation is given, b P ≤ 0.001 compared with high sodium, c P ≤ 0.05 compared with high sodium, d P ≤ 0.05 compared with C.
T-tests were used to test inter-group differences. Paired variables T-tests were used to compare data during the low and liberal sodium situation in each individual. A two sided P-value of less than 0.05 was considered to be significant. Bivariate correlation analysis was performed to analyze for a possible relation between daily insulin dose and the response to sodium restriction.

**Results**

**Patient characteristics**

Table 1 shows the subject characteristics on both study days. The average duration of diabetes was 12.3 ± 5.5 years, with a mean age of onset of 15.8 ± 6.5 years. During liberal sodium intake, BMI was 23.7 ± 2.2 kg/m² in diabetic patients and 22.2 ± 3.3 kg/m² in control subjects (NS). During low sodium diet both groups showed a non-significant decrease in BMI (DM 23.5 ± 2.2 kg/m², controls 21.8 ± 3.3 kg/m², NS). Mean HbA1c concentration was...
7.4 ± 0.5 % in the diabetic group, reflecting good metabolic control. Compliance to the low as well as to the liberal sodium diet was defined by a sodium excretion of < 60 mmol/24hr for low and > 180 mmol/24hr for the liberal sodium diet. The values for UNaV show, that compliance was satisfactory in both groups. During liberal sodium intake, PRA was similar in both groups. During low sodium, PRA increased similarly in each group.

**Systemic and renal hemodynamic effects of sodium restriction**

Mean arterial blood pressure (MAP) was similar in both groups during liberal sodium intake. The decreases in MAP during low sodium diet were not significant in either group (Table 2).

During liberal sodium, ERPF and GFR were similar in diabetic patients and the control subjects. Accordingly, FF was similar as well. Sodium restriction induced a significant and similar decrease in ERPF in both groups (-6.8± 8.7% in diabetes, -6.5± 6.9% in controls, p<0.001 vs. liberal sodium for both groups, Table 2, Fig.1). Furthermore, sodium restriction induced a significant decrease in GFR in the normal subjects ( -7.6± 9.3%, p<0.001 vs. liberal sodium, Fig.1). In the diabetic subjects however, sodium restriction did not result in a consistent reduction of GFR (Fig.1). As a result, during low sodium GFR was significantly higher in the diabetic patients than in the control subjects (p<0.05, Table 2).

Consequent to the responses of MAP and ERPF, renal vascular resistance (RVR) increased similarly during low sodium diet in diabetic subjects and controls (by 18.0± 16.8% and 17.8± 14.4% respectively, p<0.001 vs. liberal sodium, Table 2, Fig.1). In control subjects, the decreases in ERPF and GFR during sodium restriction were proportional, as indicated by the virtually unchanged filtration fraction (Table 2, Fig.1). In the diabetic subjects however, filtration fraction (FF) was significantly increased by low sodium (increase 4.1± 8.4%, p<0.05 vs. liberal sodium). As a consequence, during low sodium, FF was significantly higher in diabetic patients than in control subjects (p<0.05, Table 2).

No associations between daily insulin dose and the responses to sodium restriction were detected: the correlation coefficients between insulin dose and changes in GFR and FF were 0.1 and 0.2, (both NS), respectively.
Discussion

The present study demonstrates that a short-term moderate sodium restriction induces relative hyperfiltration in normotensive, normoalbuminuric type 1 diabetic patients.

The diabetic subjects were tested using euglycemic clamp, with low dose insulin infusion. Insulin is known to stimulate sodium retention, vasodilation and sympathetic activity (10;11;22-24), which could have influenced our results. 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 RAS-activation (25-28). The low dose of insulin that we used provides stable peripheral insulin concentrations of approximately 30 mU/l (29), 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 a reasonably well-regulated diabetic patient during daily life. The healthy control subjects did not receive insulin infusion, as insulin may exert effects on renal hemodynamics (30). Thus, as inevitable in studies comparing diabetic patients and controls, study conditions did not completely match for diabetic patients and controls. This limitation has to be taken in mind in the interpretation of the data.

In the present human studies, obviously intrarenal hemodynamics could not be measured directly. We found that low sodium diet induced a similar decrease in ERPF, with a corresponding increase in overall RVR in diabetic patients and control subjects, whereas the effect on FF was different. This suggests that sodium restriction differentially affected the balance between afferent and efferent vascular tone in diabetic patients as compared to control subjects, with a lower contribution of afferent tone in total RVR in diabetes during low sodium. There are two possible explanations for this phenomenon.

First, there may be an impaired afferent glomerular vasoconstrictor function in diabetes mellitus (31;32). These afferent abnormalities have been attributed to alterations in tubuloglomerular feedback in the diabetic state. Proximal tubular reabsorption of sodium is increased in experimental as well as in human type 1 diabetes, as shown by lithium clearance studies (33-35). These studies as well as recent animal data have demonstrated that by this increased proximal reabsorption, distal tubular delivery of sodium decreases, thereby deactivating the tubuloglomerular feedback signal, resulting in afferent vasodilatation (34;36). By this mechanism dietary sodium restriction may paradoxically aggravate glomerular hyperfiltration in uncomplicated diabetes mellitus (36).

Second, exaggerated activation of the RAS should be considered. However, we found
no differences in circulating PRA, as both groups showed a similar rise in PRA during sodium restriction. Increasing evidence suggests the existence of an intrarenal RAS, that acts independently from the systemic RAS (37,38). A study in type 1 diabetic patients with nephropathy revealed an enhanced renal vasodilator response to the administration of ACE inhibitors as well as to AngII antagonists even when PRA was low, suggesting intrarenal RAS-activation in diabetic nephropathy (39,40). Furthermore, hyperglycemia has been associated with intrarenal RAS activation in healthy humans. Captopril enhances the renal vasodilatation induced by hyperglycemia without alteration of circulating PRA, suggesting intrarenal RAS-activation by hyperglycemia (27). Because the RAS has a predominant efferent vasoconstrictor effect (41), a more pronounced activation of the intrarenal RAS in the diabetic patients in the current study could be an explanation for the observed difference in the balance between afferent and efferent vascular tone.

Thus, failure of adequate afferent glomerular autoregulation, and/or excessive efferent glomerular arteriolar vasoconstriction due to an overproduction of AngII may explain the relationship between lowering sodium intake and the increased filtration fraction. As the latter may be the reflection of increased intraglomerular pressure, sodium restriction as such may induce an unfavorable renal hemodynamic response in diabetes.

In experimental diabetes, studies on the renal effects of sodium restriction yielded conflicting results. One group reported that diabetic hyperfiltration was corrected by sodium restriction (16), while others found an increase in renal hyperfiltration (18). The renal effects of sodium restriction in human diabetes were examined in only two previous reports (19,20). In nine normoalbuminuric patients, GFR and ERPF were reduced by lowering sodium intake, with no change in FF (20). Values of ERPF and GFR were higher than ours on both studied conditions, which may be due to a longer diabetes duration or higher HbA1c concentrations. There were however no healthy control subjects in this study, so a direct comparison with our findings cannot be made.

Miller found that restriction of dietary sodium to 20 mmol/day induced a fall in RVR, accompanied by a rise in ERPF as well as GFR, in spite of an appropriate rise in PRA in type 1 diabetic patients. In the healthy control group, ERPF and GFR were unaltered (19). Thus, in accord with our data, diabetic patients in that study responded differently to low sodium than controls, with relative hyperfiltration during low sodium. At variance with our findings, the hyperfiltration during low sodium was due to hyperperfusion; i.e. a rise in renal blood flow with a fall in RVR. It might be that the less rigorous metabolic control (limit for inclusion was a HbA1c <10%, versus 8.5% in our study), and the concomitant hyperfiltration already present during liberal sodium in that study resulted in a greater
propensity to renal vasodilation (27;42). However, it might also be that differences in experimental setup account for the differences in the effects of low sodium on renal perfusion, as suggested by the differences in effect of low sodium on RVR in the healthy controls as well. In our control subjects, in accord with earlier findings (43;44), RVR was increased by low sodium. This renal vasoconstrictive response was shown to be mediated by the RAS (43;44). Such a renal vasoconstrictive response was absent in the healthy controls in Millers’ study. The reason for the discrepancy is unclear, but it could be speculated that the less pronounced waterloading in our protocol allows for more accurate detection of RAS-mediated renal responses (45).

What could be the implications of our findings? Our data suggest that sodium restriction may not be suitable for prevention of nephropathy. Our data, however, were obtained after only one week of sodium restriction, and thus require confirmation after long term sodium restriction. In addition, early hyperfiltration as described in the literature usually occurs during liberal sodium, in a non-normoglycemic condition. The interaction between glycemia and sodium restriction thus needs further exploration. Moreover, the interindividudal differences in the renal response to sodium restriction in the diabetics indicate that individual factors will have to be considered in recommendations for an optimal sodium intake. Finally, hyperfiltration often predicts nephropathy, but not inevitably so (4;46-48), thus the pathogenetic significance of our findings for the likelihood of later nephropathy remains to be investigated. In this respect it would be of interest to investigate the renal response to sodium restriction in patients with micro-albuminuria. Our data do not implicate that sodium restriction should be discarded in diabetic patients. RAS-blockade by ACE-inhibition or AII-antagonists is one of the main measures to prevent diabetic nephropathy. In non-diabetic subjects sodium restriction enhances the renoprotective effects of RAS-blockade (49;50). Thus, it would be worthwhile to investigate the effects of sodium restriction combined with RAS-blockade in diabetics as well.

In summary, short-term moderate sodium restriction induces relative hyperfiltration (but not hyperperfusion) in uncomplicated, normoalbuminuric type I diabetic patients. The findings are compatible with an altered balance between the afferent and efferent vascular tone, associated with increased intraglomerular pressure, elicited by sodium restriction. Further mechanistic studies would be needed, however, to explore the intrarenal mechanisms of this response to low sodium. Moderate sodium restriction as such may be unsuitable as a preventive approach in diabetes, but long-term studies, and studies in albuminuric patients are needed to substantiate this assumption.
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